R. C. Hughes.

STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE)

R. CHESTER HUGHES

Ann Arbor 1926 Michigan

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PREFACE

This volume contains reprints of seven distinct articles originally published separately in various biological journals. The several articles constitute parts of an extensive series of papers, being published under the same general title, by Professor George R. LaRue, his students, and associates, on researches done in the Department of Zoology and Biological Station of the University of Michigan.

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. IX. *NEASCUS VAN-CLEAVEI* (AGERSBORG)

R. CHESTER HUGHES

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. IX. *NEASCUS VAN-CLEAVEI* (AGERSBORG)*

R. Chester Hughes
University of Michigan, Ann Arbor, Mich.

Introduction

The purpose of this paper is to clear up the literature concerning, and to present a morphological account of *Neascus van-cleavei* (Agersborg), a species of strigeid metacercaria, indigenous to this country and closely related to the European *Neascus cuticola* (von Nordmann).

The occurrence in North America of a parasite classified by the respective authors as *Diplostomum cuticola* (von Nordmann) Diesing has been reported by Leidy (1856: 45), Linton (1898: 513), Stafford (1904: 494), Cooper (1915: 191) and Ward (1918: 411). According to these authors, *inter se*, it occurs in cysts in the mesenteries, kidneys, and liver and on the heart of *Eupomotis gibbosus* (L.) *Lepomis auritus* (L.), *Helioperca incisor* Cuv. and Val., *Ambloplites rupestris* (Raf.), *Notropis atherinoides* (Raf.), *Notropis cornutus* (Mitch.), *Semotilus atromaculatus* (Mitch.), *Micropterus dolomieu* Lac., and *Boleosoma nigrum* (Raf.), from Canada to Iowa and Missouri. Each of these reports was accompanied by a brief description. Linton's report, based on a study of specimens, Nos. 4842, 4843 and 4844 U.S.N.M., was illustrated by ten figures.

Agersborg (1926), apparently unaware of the foregoing notices and indeed of any literature germane to the subject of strigeid metaceriae, reported the occurrence of a parasite which he described as a new species, *Diplostomum van cleavei*, found encysted or free in the body-cavity and on the liver of *Notropis anogenus* Forbes collected near Urbana, Illinois. Although Dr. Agersborg confused anterior and posterior ends and fore- and hind-bodies of the organism and made a number of other comparable errors in his description it seems probable that he dealt with the same species previously reported by the authors cited. For reasons which are presented in subsequent pages I am convinced that these several reports have to do with a species of strigeid metacercaria, belonging to the larval group *Neascus* mihi, which is distinct from *Neascus cuticola* (von Nordmann) of Europe. Since no detailed study of this form has been made, other than that of Agersborg, which, as already suggested, is unfortunately seriously

* Contribution from the Department of Zoology and the Biological Station, University of Michigan. This is the ninth of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

confused, I feel justified in redescribing it. Dr. Agersborg's paper will be discussed following the description of my material.

Materials and Methods

In November 1926 a number of specimens of *Eupomotis gibbosus* (L). *Xenotis megalotis peltastes* Cope, and *Ambloplites rupestris* (Raf.) from the Huron River near Ann Arbor, all less than 100 mm. in length, were examined and found to be infected with this parasite. In these instances the encysted worms were found only in the liver, in numbers varying from 10 to more than 100. I also had for examination 10 heavily infected specimens of *Helioperca incisor* Cuv. and Val. about 8 inches long picked up on the beach of Gilead Lake, Branch Co., Michigan, June 12, 1927, by Mr. T. Huxley Langlois. The cysts occurred chiefly in the liver, a few in the mesenteries and on the heart. From one individual 185 worms were taken. On June 16, 1927, 4 specimens were taken from 1 *Eupomotis gibbosus* and 10 from 1 *Ambloplites rupestris*, both from the Huron River, Ann Arbor. One out of 12 adult specimens of *Semotilus atromaculatus* (Mitch.) from the Drainage Ditch of Crystal Lake, Urbana, Illinois, yielded 21 cysts which were mostly in the body cavity attached to the peritoneum; a few were in the liver. Four out of 25 adult *Hyborhynchus notatus* Raf. from the same locality were infected. Altogether 20 specimens were taken from the mesenteries of these fishes. The fishes from Urbana were collected in the early part of June 1927 by Mr. J. Lawrence Griffith. In August 1927 Mr. Langlois found several specimens in the liver of 1 *Micropterus dolomieu* Lacépède at Douglas Lake, Michigan. The specimens above mentioned were studied both alive and preserved. I have also studied preserved specimens from *Helioperca incisor* collected by Miss L. J. Hall from Douglas Lake and by Mr. Langlois from Fife Lake, Grand Traverse Co., Mich., both during the summer of 1926. The blue-gills of the latter lake were reported to be dying in large numbers and the dead fishes examined carried enormous infections of the present species. I have also examined the specimens (Nos. 4842, 4843, and 4844 U.S.N.M.) which were reported by Linton (1898) and those collected by Professor Van Cleave from *Helioperca incisor* at Havana, Illinois, and regarded by Agersborg (1926: 20) to be identical with his type material, and three specimens from *Fundulus diaphanus* (Washington, D. C.), mounted *in toto* (slides No. 491 of the U. S. Bureau of Animal Industry Coll. No. 1717) which also belong to the present species. For purposes of comparison I have made use of several preserved specimens of *Neascus cuticola* from *Leuciscus* sp., from the vicinity of München, Germany, which had been presented to Professor La Rue by Professor L. Scheuring. These specimens were stained as outlined below, some mounted *in toto* and others sectioned.

The parasites on which this description is primarily based were carefully studied alive. Many of the details of the excretory system can be worked out only in living material, and the spination and sub-cuticular muscle layers are more easily studied in living specimens. The application of pressure was often found helpful in bringing out details, but measurements were taken from specimens not subjected to pressure.

Sublimate-acetic was generally used for fixation. For whole mounts regressive staining, and for sections progressive staining *in toto* in a weak solution of Ehrlich's acid haematoxylin in 70% alcohol was found to be very satisfactory, particularly because it eliminated the necessity of transferring the specimens through a long series of grades of alcohol, a process which often caused the thin walls of the hind-body to collapse. Sections of 8 microns were made in the three cardinal planes.

Description of Parasite

This description is based primarily upon specimens taken from *Eupomotis gibbosus* L. from the Huron River near Ann Arbor, collected in November, 1926. Noticeable variations in specimens from other localities and other hosts will be pointed out.

The Cyst. The cyst is usually much larger than the enclosed parasite so that the latter is generally not rolled up or folded in any way (Fig. 1). Cysts taken from the mesenteries are usually relatively smallert han those from the liver, but even in these smaller cysts the parasite is not crowded. The thin wall of the cyst is composed of two layers, an outer of host origin and an inner of parasite origin. The outer layer is usually attached to the serosa of the host by a slender pedicel. This was observed and figured by Linton (1898: 513 and Pl. XLII fig. 2). The position of this attachment is marked on the inner cyst by a circular scar (Fig. 1, *a*). The outer cyst often shows a number of transverse wrinkles (Fig. 1). The cyst is easily opened without injury to the parasite. It contains, surrounding the parasite, a heavy colorless sticky gelatinous fluid in which a quantity of excretory granules are suspended. Cysts taken from freshly killed specimens of *Eupomotis gibbosus*, in Nov. 1926, not under pressure, varied in length from 1.05 mm. to 1.45 mm. and in width from 0.65 mm. to 0.75 mm. Cysts from specimens of *Helioperca incisor*, taken from Gilead Lake in June 1927, were considerably larger, 10 cysts varying in length from 1.59 mm. to 1.8 mm. (averge 1.68 mm.) and in width from 0.6 mm. to 0.99 mm. (average 0.81 mm.). Although the cysts in this case contained living parasites, the fish from which they were taken had already begun to decay before they were collected and placed on ice. It seems very probable that the cysts and parasites were somewhat larger than normal due to the absorption of water. It was observed that specimens taken from freshly killed fishes, when placed in water, increased rapidly in size due to imbibition of water. This is not surprising since the

gelatinous content of the cyst and the excretory spaces of the parasite might be expected to have a high osmotic pressure.

The Parasite. Freed from their cysts and not subjected to pressure 5 specimens from *Eupomotis gibbosus* in Nov. 1926 varied in length from 0.91 mm. to 1.2 mm. (average 0.99 mm.). Five examples taken from the specimens of *Helioperca incisor* mentioned above were from 1.56 mm. to 2.24 mm. long (average 1.87 mm.). The following table shows the variation in the lengths of preserved specimens taken from different hosts and from different localities. It may be pointed out that both of the extreme limits of variation among the specimens measured occur in those taken from *Helioperca incisor* from Gilead Lake.

TABLE SHOWING VARIATION IN THE LENGTH OF PRESERVED SPECIMENS OF *Neascus van-cleavei* FROM DIFFERENT HOSTS AND DIFFERENT LOCALITIES.

Number of parasites measured	Host	Locality	Date	Length in millimeters		
				Minimum	Maximum	Average
10	*Eupomotis gibbosus*	Ann Arbor, Michigan	Nov. 1926	0.56	1.06	0.73
10	" "	Ann Arbor, Michigan	June 1927	0.51	0.70	0.64
10	*Helioperca incisor*	Fife Lake, Michigan	Summer 1926	0.56	1.06	0.81
6	" "	DouglasLake Michigan	Summer 1926	0.70	0.88	0.80
10	" "	Gilead Lake, Michigan	June 1927	0.40	1.21	0.70
5	*Ambloplites rupestris*	Ann Arbor, Michigan	June 1927	0.60	0.63	0.62
10	*Semotilus atromaculatus*	Urbana, Illinois	June 1927	0.74	0.95	0.82
10	*Hyborhynchus notatus*	Urbana, Illinois	June 1927	0.64	1.02	0.78

The parasites within the cyst were often seen slowly lengthening and contracting the body, particularly in response to gentle pressure on the cyst wall. Even when removed from the cysts the parasites are, however, very sluggish. The movement consists merely of extending and contracting the various parts of the body, and no actual locomotion is accomplished. The oral sucker and acetabulum are not used for attachment, so far as observed. The leech-like movement so frequently noted in some diplostomes and in *Neascus ambleplitis* mihi (Hughes 1927: 251) was never observed.

A constriction divides the body of the parasite into two well defined regions, the fore- and hind-bodies (Figs. 1, 4, *c*). The constriction is discontinuous across the dorsum of the body, so that the two body regions are connected dorsally by a ridge (Fig. 1, *dr*). The axis of the body is often, but not always, slightly flexed dorsally in the region of the constriction.

The fore-body, generally two or three times as long as the hind-body, is relatively thin, elliptical in outline, and somewhat spoon-shaped with the dorsal side convex, and without lateral sucking cups. Its shape is very changeable in accordance with the movements of the body. Contracted it becomes rather thick, cordate, broadened anteriad, with a distinct notch in the anterior margin in the region of the oral sucker. Elongated it becomes thin and ribbon-like with an apical cone in the region of the oral sucker. The fore-body is bent abruptly ventrad near its posterior end and the postero-lateral margins of the fore-body are raised forming a shallow concavity in which the acetabulum and hold-fast organ lie. It is to the dorsal surface of this portion of the fore-body that the hind-body is attached. The entire surface of the fore-body is spinous, except the acetabulum. In 5 living specimens removed from their cysts and free from pressure the length of the fore-body varied from 0.52 mm. to 0.89 mm. (average 0.68 mm.) and the width from 0.30 mm. to 0.42 mm. (average 0.35 mm.). In 8 whole mounts the length varied from 0.30 mm. to 0.41 mm. (average 0.35 mm.), and in width from 0.28 mm. to 0.44 mm. (average 0.35 mm.).

The hind-body varies in shape from conical to almost spheroidal. The latter condition obtains when the hind-body is turgid with excretory substances. Sometimes the hind-body is slightly bilobate in appearance, due to the presence of a shallow median groove which goes around the posterior end and along both the dorsal and ventral surfaces. Such specimens also show a pair of ventro-lateral grooves in the anterior half of the hind-body. The position of these grooves indicates the locus of the internal septa between the large excretory spaces of the hind-body (Fig. 8) and their presence indicates extreme turgidity with excretory fluids. If the parasite is stimulated by gentle pressure with a needle or with a little acid it discharges the contents of the excretory spaces and the hind-body becomes more and more conical as deflation proceeds. The hind-body is less active than the fore-body, and variations in its shape are related more frequently to the content of the excretory spaces than to states of contraction. In 5 living specimens removed from their cysts and free from pressure the length of the hind-body varied from 0.22 mm. to 0.40 mm. (average 0.31 mm.) and the width from 0.25 mm. to 0.39 mm. (average 0.30 mm.). In 7 specimens mounted *in toto* the length of the hind-body varied from 0.22 mm. to 0.33 mm. (average 0.28 mm.) and the width from

0.25 mm. to 0.38 mm. (average 0.31 mm.). These figures in comparison with those in the preceeding paragraph indicate that the hind-body shrinks much less in preservation than the fore-body. This may be due partly to the fact that the excretory content of the hind-body is gelatinous and probably does not diffuse readily through the body-walls, whereas in the fore-body the excretory fluid is thin and watery, and partly to the fact that the musculature of the fore-body, being stronger than that of the hind-body, contracts more in fixation, thereby, forcing more excretory fluid into the hind-body.

The hold-fast organ, in specimens freshly removed from the host and not distorted by the imbibition of water, is represented by a slight elevation, circular in outline and located on the ventral side near the posterior end of the fore-body. Its surface is marked by a median longitudinal slit, the external opening of a relatively simple cavity, the glandular walls of which are not conspicuously folded in the complicated manner seen in many strigeid metacercariae. Concomitantly with the absorption of water by the organism the spinous inner surfaces of the hold-fast organ may be protruded through the external opening. When partially everted the organ has the form of two short longitudinal folds separated by a deep cleft (Fig. 4); completely everted the cleft disappears and the organ is toad-stool shaped, nearly circular in outline (Fig. 1). The hold-fast organ was seldom observed to move; its musculature seems to be rather weak in comparison with that of *Neascus ambloplitis*. In 5 living spcimens, free from pressure, the diameter of the hold-fast organ varied from 0.12 mm. to 0.15 mm. (average 0.14 mm.). In living specimens the hold-fast organ is nearly circular in outline but in preserved specimens it is broader than long due to greater shrinkage in the longitudinal axis. In 5 whole mounts the length of the hold-fast organ varied from 0.067 mm. to 0.088mm. (average 0.074) and the width from 0.081 mm. to 0.105 mm. (average 0.095 mm.). The hold-fast gland (Fig. 4, *hfg*) is a transversely elongated mass of intensely heavily staining cells located adjacent to the postero-dorsal wall of the hold-fast organ.

The oral sucker, terminal in position and circular in outline, is a small and apparently weak structure, since it seldom shows any sign of movement. In each of 4 living specimens, free from pressure, the oral sucker measured 0.031 mm. in diameter. In 5 whole mounts it varied from 0.018 mm. to 0.028 mm. (average 0.020 mm.) in diameter.

The acetabulum, located a short distance in front of the hold-fast organ and about two-thirds the length of the fore-body from the oral sucker is also apparently weak in this stage of its development. In 4 living specimens, free from pressure, its diameter was 0.046 mm., 0.062 mm., 0.062 mm., and 0.062 mm. respectively; about twice as large as the oral sucker. Like the hold-fast organ, the acetabulum in preserved specimens is broader

than long. In 5 whole mounts the length varied from 0.042 mm. to 0.053 mm. (average 0.046 mm.) and the width from 0.060 mm. to 0.070 mm. (average 0.064 mm.).

The structures referred to by Leidy (1856: 45) as "generative apertures" were the acetabulum and hold-fast organ. The "generative apertures" of Linton (1898: 513) represent the opening of the hold-fast organ. In these errors the authors cited followed examples set by Diesing (1850: and 1858) in his descriptions of Tylodelphys and Diplostonum.

The bursa copulatrix, postero-dorsal in position, consists of an antero-medial and a pair of postero-lateral muscular folds, embracing a Y-shaped cavity communicating with the exterior through the genital pore (Fig. 4, *bc*, *gp*). When extruded, a condition rarely seen, it appears as shown in Fig. 1, *bc*. Probably the eversion of the hold-fast organ, acetabulum and the bursa copulatrix is brought about by pressure due to the absorption of water, rather than by any normal activity of the larva. In 5 mounted specimens the diameter of the bursa copulatrix, not everted, varied from 0.053 mm. to 0.088 mm. (average 0.068 mm.).

The digestive tract comprises the usual parts. The oral sucker has already been described. The prepharynx is very short. The pharynx is small, elongate. In a living specimen it was 0.023 mm. long. In 5 specimens mounted *in toto* it was from 0.028 mm. to 0.039 mm. (average 0.033) mm.) in length and from 0.018 mm. to 0.028 mm. (average 0.021 mm. in width. The slender oesophagus is short. The caecal furcae diverge at a small angle until they come to lie ventral to the intra-lateral excretory vessels (Fig. 6, *int*). They extend beneath the intra-lateral excretory vessels, nearly parallel to each other, back to the region between the acetabulum and hold-fast organ where they diverge more widely. At the level of the constriction between the two body-regions they bend mesad and ventrad. In the hind-body they come to lie close to the ventral cuticle in the septa between the ventro-lateral and dorso-lateral excretory vessels. They terminate blindly in the regions lateral to the bursa copulatrix. At the bifurcation of the oesophagus the caeca are slender. They become gradually broader as they proceed posteriorly, reaching a maximum diameter at about the middle of the hind-body. In living specimens no solid substances were observed in the caeca.

The nervous system comprises a supra-pharyngeal commissure and two pairs of branches, one pair extending anteriorly from the ends of the commissure and the other posteriorly as large trunks which were traced back to the regions lateral to the hold-fast organ where it was not possible to trace them further. The posterior branches lie, for the greater part of their length, ventral to the primary lateral excretory vessels (Fig. 6, *pn*) and just beneath the ventral cuticle. The posterior nerves give off laterally in the anterior part of the fore-body several pairs of branches. The finer details of the nervous system were not worked out.

The entire body is provided with sub-cuticular circular and longitudinal muscle fibers. These fibers in the hind-body are much thinner and weaker than in the fore-body. The fore-body is also provided with an additional set of large longitudinal fibers situated more deeply in the peripheral parenchyma (Fig. 6, *imf*), which do not extend into the hind-body. These inner longitudinal fibers are much larger in size and fewer in number than the outer fibers, and are so conspicuous that they give the fore-body a characteristic longitudinally striated appearance, a condition observed by Linton (1898: P1. XLII, fig. 1), both in living and preserved specimens. The fact that the fore-body shrinks so much in the longitudinal axis in preservation may be due to the strong contraction of these fibers. The genital and excretory pores are provided with sphincter muscles.

The reproductive system comprises three, more or less well defined, heavily-staining cell masses, each surrounded by a thin membrane, located in the septa between the large excretory spaces in the anterior half of the hind-body (Fig. 4, *rf*). Two of these are ventrally situated as a pair in the septa between the ventro-lateral and dorso-lateral excretory vessels, just posterior to the constriction. Posterior to these is the third, transversely elongate, inversely V-shaped fundament, the apex of which extends dorsally into the median septum between the dorso-lateral excretory vessels and the legs ventro-laterally into the septa between the ventro-lateral and dorso-lateral excretory vessels. These septa and excretory vessels are shown in Fig. 8. In some of the larger specimens mounted *in toto* more than three fundaments may be seen, but in no case has differentiation proceeded sufficiently to permit an interpretation of the respective fundaments in terms of the mature reproductive system. Such an interpretation would require the study of a developmental series, obtained from the definitive host, as yet unknown.

A pair or strands or cords of large cells with large nuclei (Fig. 7, *vc*), thought to be the fundaments of vitellaria, are associated with the intestinal caeca. The anterior ends of these cords are at the level of or at a variable distance in front of the acetabulum, according to the size of the individual. These cords, anteriorally small, ventral to the caeca and cylindrical in shape, become prominent at the level of the hold-fast organ where they are ventro-lateral to the caeca and trough-shaped with their concave surfaces applied to the caeca. Posteriorly the cords become attenuated, more cylindrical, and shifted mesially beneath the caeca until their posterior ends come to lie adjacent to the medio-ventral surfaces of the caeca, near the distal ends of the latter.

In many respects the excretory system of the present species closely resembles that of *Neascus ambloplitis* mihi (Hughes 1927: 251). As far as applicable the same terminology is used in the present description. According to Faust (1922: 80) and Hughes (1927: 253) the excretory

system of the holostome larva is comprised of two parts, the primary excretory apparatus consisting of flame cells and associated tubules and the reserve bladder. As in *N. ambloplites* the *primary excretory apparatus* is greatly obscured by the thickness of the animal and the excessive development of the reserve bladder. Of the said primary excretory apparatus a few scattered flame cells and tubules were observed, but not in sufficient detail to give any definite idea of their arrangement. The excretory system was studied both in living material and in sections. The former method was much more fruitful since in preservation many of the finer details are lost. Sections were, however, indispensable for the study of certain larger features.

The reserve bladder comprises an intricate network of large and small vessels, the arrangement of which, although variable, shows a high degree of regularity. In the fore-body these vessels contain a limpid transparent fluid, in which float calcareous concretions of various sizes. This fluid with the contained granules surges about from one part of the body to another in response to muscular movements. In the hind-body the excretory vessels are very large, and are filled with large spheroidal masses of heavy gelatinous fluid (Fig. 5, *eg*), suspended in a thin fluid, apparently the same as that in the vessels of the fore-body. Concretions occur both in the globules of viscous fluid and in the surrounding thin fluid. This thinner fluid, in flowing from the main trunks of the fore-body through the hind-body to the excretory pore often forms definite channels among the denser globules, which do not surge about, but move rather slowly back and forth in limited space, in response to body movements. When in motion these droplets become elongated in the direction of movement. Under pressure of a cover glass they exude from the excretory pore. Such droplets in contact with the water remain intact for some time, but finally disintegrate. It was thought at first that this substance was an oil or fat, but tests with Sudan III solution and with osmic acid gave negative results. If the hind-body of a living specimen is teased apart with needles the dense fluid gums on the needle points in the manner characteristic of crystalline lens material. The substance apparently is not soluble in alcohol or in xylol for in sections of specimens fixed in sublimate-acetic, stained *in toto* in Ehrlich's acid haematoxylin, dehydrated in absolute alcohol, cleared in xylol, and embedded in paraffin, the excretory spaces are filled with a more or less homogenous pale blue substance.

The principal vessels of the reserve bladder are as follows. The *median dorsal vessel* (Fig. 5, *mdv*) extends from the region immediately back of the oral sucker to the posterior margin of the hold-fast organ. This vessel does not divide to form rings about the acetabulum and hold-fast organ as in *Neascus ambloplitis* but continues as a single vessel dorsal to each of the structures named. Just behind the oral sucker the median dorsal

vessel is connected with the inner pair of longitudinal vessels by four pairs of *anterior transverse commissural vessels* (*atcv*), and just behind the acetabulum by one pair of *posterior transverse commissural vessels* (*ptcv*). This inner pair of longitudinal vessels corresponds to the *intra-lateral vessels* (*ilv*) of *N. ambloplitis*. The present species differs again from *N. ambloplitis* (Hughes 1927: Pl. 6, Fig. 4) in that the transverse commissural vessels are of two distinct groups and in that they communicate primarily with the intra-lateral rather than with the primary lateral vessels. The two species agree, however, in that the second pair of lateral longitudinal vessels, the *primary lateral vessels* (*plv*), is the largest, external to which on either side, is an irregular network of spaces, which in the present species may be referred to as the paired *extra-lateral networks* (*elnw*). The primary lateral vessels are connected with the respective intra-lateral vessels and extra-lateral networks by numerous, more or less uniformly distributed, anastomoses. Posteriorly, the lateral vessels on either side are collected into two vessels, a large inner *lateral collecting vessel* (*lcv*) and a small outer *marginal vessel* (*mv*). Each of these vessels unites with the corresponding vessel of the other side. The marginal vessel extends only through the posterior lip of the fore-body, differing in this respect from *N. ambloplitis* in which the closed marginal vessel runs along the entire margin of the fore-body.

The broad, short *median ventral vessel* (*mvv*) formed by the union of the lateral collecting vessels is situated between the hold-fast organ and the constriction. It divides immediately upon passing into the hind-body giving origin to a pair of large *ventro-lateral vessels* (Figs. 5, 8, *vv*). The median dorsal vessel (*mdv*) bifurcates just behind the hold-fast organ giving rise to a pair of *dorso-lateral vessels* (Figs. 5, and 8, *dv*). These are abruptly expanded as they enter the hind-body, occupying most of the space in the hind-body, being separated from each other and the smaller ventro-lateral vessels only by delicate septa, except in the regions of the reproductive fundaments and the bursa copulatrix. Postero-dorsally they unite and communicate abruptly with the terminal excretory pore. Anteriorly the ventro-lateral vessels are connected to the dorso-lateral vessels by two pairs of anastomoses, and to each other just in front of the bursa copulatrix by a single anastomosis. They empty into the dorso-lateral vessels in the regions lateral to the bursa copulatrix. The present species differs markedly from *N. ambloplitis* in respect to the arrangement of the vessels of the reserve bladder in the hind-body. In the present species these vessels are very much larger and display a much simpler arrangement. Nothing comparable to the muscular bladder or the semi-circular commissural vessels of *N. ambloplitis* is present. In *N. ambloplitis* not the least indication of heavy gelatinous fluids in the excretory spaces was ever observed.

Discussion

The name Diplostomum was applied by von Nordmann (1832) to forms which he found in the eyes of fishes. Although von Nordmann considered these forms to be adults it has since been demonstrated that they are matacercariae. The use of the term metacercaria ("meta-cercaire") was proposed by Dollfus (1912: 87) as follows:" . . . . . c'est celui de Trématode dans la période de son évolution qui s'étend entre la fin de sa vie libre à l'état de cercaire issue du sporocyste et son établissement dans l'hote definitif où il acquiert sa maturité sexuelle." Brandes (1888: 50) used the name Diplostomum, not of von Nordmann, to designate a new genus for adults. This usage has been followed by a number of authors but in most literature of recent years the use of the name Diplostomum has been restricted to larval forms. Agersborg (1926: 19) referred his new species to the genus "Diplostomum (von Nordmann) Brandes (1888)" thereby confusing the two uses of the term. It is obvious that Agersborg thought he was dealing with an adult form since he listed it with ten species of adult trematodes all belonging to the genus Diplostomum Brandes and called especial attention to the fact that all of the previously described species were parasitic in the intestines of their respective hosts, whereas his form lived in the coelomic cavity or on the liver.

Efforts to secure type or cotype material of *Neascus van-cleavei* from Dr. Agersborg have been futile. An effort to secure specimens of *Notropis anogenus*, the type host, from the neighborhood of Urbana, the type locality, was also unsuccessful. Agersborg (page 18) reported that this species of fish occurred in great abundance in September and October of 1921 decreasing in numbers during the winter. Information is at hand from the Illinois State Natural History Survey Division to the effect that it has no records of the occurrence of *N. anogenus* from the vicinity of Urbana or from any other points so far south. It seems probable that the species of fish from which Agersborg obtained his parasites was *Hyborhynchus notatus* which is common in the vicinity of Urbana and which, superficially, closely resembles *Notropis anogenus*. The reader will recall that among my specimens were some from *H. notatus* from the Drainage Ditch of Crystal Lake, Urbana.

In discussing the effect of this parasite on the host Agersborg(page27) makes the statement that "only three trematodes are sufficient to upset metabolism." He kept his fishes in aquaria and the symptoms observed were probably brought about by the conditions of the environment, to which the fishes were not able to adjust themselves. This argument is supported by the fact that Agersborg (page 19) found that by aerating the water the discomfiture of the fishes was relieved. I have often examined fishes which showed no outward signs of disease and found them heavily infected with this species.

In his description and figures Agersborg made the following misinterpretations: anterior for posterior, hold-fast organ for acetabulum, acetabulum for genital papilla, parts of the bursa copulatrix for retractile buccal loops, mucous extrusions for spines, median dorsal excretory vessel for alimentary canal, oral sucker for posterior sucker, mouth for excretory pore, part of the bursa copulatrix for a giant flame cell, concretions in the excretory spaces for yolk granules, excretory pore for mouth, and other errors which I am unable to account for. Notwithstanding these errors Agersborg's figures bear a close resemblance to the specimens which I have examined. Furthermore, the specimens collected by Professor Van Cleave and determined by Agersborg (1926: 20) to be identical with his *Diplostomum van cleavei* are similar in all respects to my material.

In general the brief descriptions given by the American authors cited in the introduction agree with my findings, and a study of the specimens in the U. S. National Museum reported by Linton (1898) indicates that they are identical with my material.

Comparative Synopsis of Described Neascus Larvae

1. **Neascus cuticola** (von Nordmann).

Synonymy: *Holostomum cuticola* von Nordmann (1832: 49)
Diplostomum cuticola Diesing (1850: 306)
Diplostomum cuticola Krøyer (1852-53: 1250)
Diplostoma cuticola, Diesing, Cobbold (1860:50).
Tetracotyle cuticola (Nord.) Kowalewski (1902: 25)
Diplostomulum cuticola Faust (1917: 67)
Neascus cuticola (von Nordmann) Hughes (1927: 258)

This species was first described by von Nordmann (1832: 49). Descriptions, based on that of von Nordmann, have been published by von Nordmann (1843), Dujardin (1845: 379), Diesing (1850: 306 and 1858: 317), Moulinié (1856: 217), Waldenburg (1860: 8), Villot (1898: 541), Weltner (1899: 52), Hofer ((1904: 139) and Lühe (1909: 166). Recently the species has been studied more in detail by Scheuring and Eversbusch (1926: 44). Among these descriptions and the reports of Krøyer (1838-40: 578), Wigham (1851: 235), Pavesi (1881: 615), Kowalewski (1902: 23) and Zandt (1924: 239), *Neascus cuticola* has been listed from *Leuciscus dobula, L. rutilus, L. idus, L. vularis, L. erythrophthalmus, Gobio vulgaris, Phoxinus laevis, Perca fluviatilis, Abramis brama, A. vimba, Blicca bjoerkna, Cobitis taenia, Chondrostoma nasus, Cyprinus carpio, C. blicca, Gobio fluviatilis, Idus melanotus, Scardinius erthrophthalmus* and *Squalius cephalus* from various parts of Europe. Steenstrup (1845: 97) suggested that the four forms described by von Nordmann from the eyes of fishes, viz. *Diplostomum clavatum, Holostomum cuticola, Holostomum brevicaudatum* and *Diplostomum volvens* represent successive stages in the life cycle of a single

species. Villot (1898) advanced the idea that *N. cuticola* is the larva of *Hemistomum denticulatum* (Rudolphi). Scheuring and Eversbusch (1926) held that this assumption is untenable and were of the opinion that *Strigea cornu* (Rudolphi) is the definitive form. In neither case was the conclusion attained based upon the results of feeding experiments.

Neascus van-cleavei closely resembles *N. cuticola* in size and body form. For the purposes of comparison use has been made of the specimens sent to us by Professor Scheuring and identified by him as *Diplostomum cuticola*. This identification is corroborated by my studies. In these specimens I find that the arrangement of the excretory vessels in the fore-body (Fig. 9) is very similar to that of *N. van-cleavei*. The more important differences between the two species are tabulated as follows.

Neascus van-cleavei	*Neascus cuticola*
1. Cysts, non-pigmented, thin-walled, in peritoneum of coelem and viscera and in liver of hosts.	Cysts, black-pigmented, thick-walled, in skin, fins and muscles, etc.
2. Inner cyst elongate, so large that parasite is not folded (fig. 1).	Inner cyst spherical, closely investing parasite (fig. 3).
3. Constriction between fore- and hind-bodies discontinuous dorsally (fig. 1, *dr*).	Constriction between fore- and hind-bodies completely encircling body.
4. Fore-body coarsely striated longitudinally due to presence of large inner longitudinal muscle fibers (fig. 6, *imf*.)	Fore-body not coarsely striated longitudinally; large inner longitudinal muscle fibers apparently wanting (fig. 9).
5. Acetabulum about twice as large as oral-sucker.	Acetabulum just a little larger than oral-sucker (Scheuring and Eversbusch, 1926: 47).
6. Intestinal caeca in hind-body, relatively small and located close to ventral body-wall (fig. 8. *int*).	Intestinal caeca in hind-body large and far removed from body-wall (figs. 10 and 11, *int*).
7. Presence of cords of cells, interpreted as fundaments of vitellaria, associated with digestive caeca (fig. 7, *vc*).	Such cords wanting. According to Scheuring and Eversbusch (1926: fig. 1) vitellaria are represented by a pair of rounded masses in anterior part of hind-body.
8. In hind-body two distinct pairs of excretory vessels (fig. 8, *dv, vv*) relatively free from interspersed tissue strands. These are dorso- and ventro-lateral vessels. No internal vessels present.	In hind-body one pair of distinct dorso-lateral vessels in which delicate tissue strands occur, a pair of definite *internal vessels* and a net-work of ventral vessels (figs. 10 and 11, *dv, iv, vnw*).

2. Neascus brevicaudatus (von Nordmann) mihi.

Synonymy: *Holostomum brevicaudatum* von Nordmann (1832: 52)
Diplostomum brevicaudatum Diesing (1850: 306)
Diplostoma brevicaudatum Diesing, Cobbold (1860:50)
Tetracotyle brevicaudata Mataré (1910: 496)

Descriptions of this species based on the original description of von Nordmann (1832: 52) have been published by von Nordmann (1843), Dujardin (1845: 380), Diesing (1850: 306), Lühe (1909: 166) and Zandt (1924: 238). It is a rare species. The only records are three specimens

reported from the eyes of *Perca fluviatilis* by von Nordmann and a few from the eyes of *Lota vulgaris* by Zandt. It differs from *N. cuticola* as follows: it is not encysted; the axis of the body is not flexed at the juncture of the two body regions; the fore-body is distinctly heart-shaped, narrowed posteriorly; the acetabulum is smaller than the oral-sucker.

3. Neascus musculicola (Waldenburg) mihi.

Synonymy: *Holostomum musculicola* Waldenburg (1860: 12)
Diplostomum musculicola Braun (1892: 796)
Tetracotyle musculicola Braun (1894a: 167)

The original description of this species (Waldenburg 1860: 9) was paraphrased by Lühe (1909: 167). According to these descriptions and the reports of Braun (1892: 796 and 1894 a: 167) it has been found encysted in the muscles of *Perca fluviatilis*, *Scardinius erythrophthalmus*, *Abramis brama* and *Leuciscus rutilus*. *N. musculicola* differs from *N. cuticola* in that the cyst is not pigmented, the body is smaller and not folded in the cyst and the hind-body is relatively larger. According to Waldenburg the two forms are very similar and possibly identical, the differences in structure being due possibly to differences in degree of development. By a feeding experiment performed by the Ehrhardt brothers (Braun 1894 a: 167 and 1894 b: 20) it was determined that *N. musculicola* does not develop to maturity in *Corvus cornix* and *Larus ridibundus*.

4. Neascus ambloplitis Hughes

This form, found in *Ambloplites rupestris* Raf. and *Micropterus dolomieu* Lac. in Douglas Lake, Michigan, was described by Hughes (1927: 249). *N. ambloplitis* differs from *N. van-cleavei* in the following respects: it occurs in thick-walled black-pigmented tissue cysts in the fins, skin and muscles of the hosts; the inner cyst (Fig. 2) is tough, small and closely invests the parasite; the hind-body is relatively narrow, longer and more active than in *N. van-cleavei*; the fore-body is more spoon-shaped and not longitudinally coarsely striated by large inner longitudinal muscle fibers; the postero-ventral lip of the fore-body extends far forward over the hold-fast organ; the reproductive system is represented by a single pair of fundaments. Marked differences in the reserve bladder have been discussed in the description of *N. van-cleavei*.

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I wish to express my grateful appreciation to Professor George R. La Rue of the Zoology Department and Director of the Biological Station, University of Michigan, by whom this study was suggested and under whom it has been conducted.

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For the identification of fishes I am obligated to Dr. Carl L. Hubbs of the Museum of Zoology, University of Michigan.

Literature Cited

Agersborg, H. P. K. 1926.—Studies on the effect of parasitism upon the tissues. II. With special reference to a new diplostomous trematode found in the minnow, *Notropis anogenus* Forbes. Archiv f. Schiffs—und Tropenhyg. 30:18–30, 13 figs.

Brandes, G. 1888.—Die Familie der Holostomeae. Ein Prodromus zu einer Monographie derselben. Diss. 72 pp. Reudnitz-Leipzig.

Braun, M. 1892.—Vermes. In Bronn's Kl. u. Ord. d. Thier-Reichs, 4, Abt. IA. 925 pp. Leipzig.

1894a.—Zur Entwicklungsgeschichte der Holostomiden. Nach den Untersuchungen der Herren Alfred und Oscar Ehrhardt mitgetheilt. Zool. Anz., 17: 165–167.

1894b.—Idem. Zool. Centralbl., 1 (19–20):755.

Cobbold, T. S. 1860.—Synopsis of the Distomidae. J. Proc. Linn. Soc. Lond., 5: 1–56.

Cooper, A. R. 1915.—Trematodes from marine and fresh-water fishes. Trans. Roy. Soc. Can., (3) 9:181–205, 3 pl.

Diesing, K. M. 1850.—Systema Helminthum. Vol. 1. 679 pp. Vindobonae.

1858.—Revision der Myzhelminthen. Abt.: Trematoden. Sitzungsb. d. k. Akad. d. Wissensch. Math. Naturw. kl., 32 (23):307–390, 2 pls.

Dollfus, R. 1912.—Contribution a l'étude des tréma totes marins des cotes du Boulonnais. Une métacercaire margeritig-ne parasite de *Donax vittatus* Da Costa. Memoires Soc. Zool. France,25: 85–144.

Dujardin, F. 1845.—Histoire naturelle des Helminthes ou vers intestinaux. 654 pp. Paris.

Faust, E. C. 1917.—Life history studies on Montana trematodes. Ill. Biol. Monogr., 4(1): 7–120, 9 pls.

Hofer, B. 1904.—Handbuch der Fishkrankheiten. 359 pp. München.

Hughes, R. C. 1927.—Studies on the trematode family Strigeidae (Holostomidae) No. VI. A new metacercaria *Neascus ambloplitis*, sp. nov. representing a new larval group. Trans. Amer. Microsc. Soc. 46:248-267, 2 pls.

Kowalewski, M. 1902.—Materyaly do fauny helmintologieznej pasorzytniczej polskiej. 3. Sprawoz. Kom. fizyogr. Akad. Umiez. w Krakow., 36 (2):21–30.

Krøyer, H. 1838–40.—Danmark's Fiske. Vol. 2. 644 pp. Kjøbenhavn. 1852-53.—Idem. Vol. 3 (2. Afd.) pp. 705–1279. Idem.

Leidy, J. 1856.—A synopsis of entozoa and some of their ecto-congeners, observed by the author. Proc. Acad. Nat. Sc., Phila., 8:42–58.

Linton, E. 1898.—Notes on trematode parasites of fishes. Proc. U. S. Nat. Mus., 20:507–548, 15 pls.

Lühe, M. 1909.—Parasitische Plattwürmer I. Trematoden. Brauer's Süsswasserfauna Deutschlands. Heft 17. 217 pp.

Mataré, F. 1910.—Ueber eine neue Tetracotyle im Hirn von *Phoxinus laevis*. Zeitschr. f. wiss. Zool., 94:488–540. 3 figs., 1 pl.

Moulinié, J. J. 1856.—De la reproduction chez les trématodes endo-parasites. Mém. de l'Inst. Nation. genevois, 3:7–278, 6 pls.

von Nordmann, A. 1832.—Mikrographische Beiträge zur Naturgeschichte der wirbellosen Thiere. I. 118 pp. Berlin.

1843.—Sur les Helminthes, dans l'oeil des animaux supérieurs. Archives de Médecine Comparée, 1:67–113.

Pavesi, P. 1881.—Dalle mie annotazioni zoologiche. Trematode nuovo parassita d'un pesce fluviale. R. Ist. Lomb. di c. Se. Lett., (2.s.) 14:615–616.

Scheuring, L. and Eversbusch, E. 1926.—Beiträge zur Entwicklungsgeschichte von *Strigea* (*Holostomum*) *cornu* Rud. Zool. Anz., 66:41–54, 7 figs.

Stafford, J. 1904.—Trematodes from Canadian fishes. Zool. Anz., 27:481–495.

Steenstrup, J. J. S. 1845.—On the Alternation of Generations; or, The Propagation and Development of Animals through Alternate Generations: a peculiar form of fostering the young in the lower classes of animals. Translated from the German version of C. H. Lorenzen, by George Busk, 132 pp. London.

Villot, F. C. A. 1898.—L'hemistome du Martin—pêcheur. Ass. franç pour l'avance. d. sc. C. -r., 26 (2 pt.):538–542, 1 fig.

Waldenburg, L. 1860.—De structura et origine cystidum verminosarum. Diss. 32 pp. Berolini.

Ward H. B. 1918.—Parasitic flatworms. In Ward and Whipple: Fresh-water Biology. Chap. XIII: 265–453.

Weltner, W. 1899.—Ueber einen Ektoparasiten unserer Weissfishe (*Diplostomum cuticola*). Blätt. f. Aquar.-Terrar. Freunde. 10:51–52, 2 figs.

Wigham, R. 1851.—*Holostomum cuticola*. Annals and Mag. Nat. Hist. (ser. VIII) 2:235, 2 figs.

Zandt, F. 1924.—Fischparasiten des Bodensees. Centralbl. f. Backt. u. Parasitenk. (I. Abt. Orig.) 92:225–271, 33 figs.

Explanation of Figures

a—place of attachment of cyst to serosa of host
ac—acetabulum
an—anterior nerve
atcv—anterior transverse commissural excretory vessels
bc—bursa copulatrix
c—constriction between fore- and hind-bodies.
cc—calcareous concretion
dr—dorsal ridge connecting fore- and hind-bodies
dv—dorso-lateral excretory vessel of hind-body
eg—gelatinous globule of excretory fluid
elnw—network of extra-lateral excretory vessels
elv—extra-lateral excretory vessels
ep—excretory pore
gp—genital pore
hf—hold-fast organ
hfg—hold-fast gland
ilv—intra-lateral excretory vessel
imf—inner longitudinal muscle fiber
int—intestine
iv—inner excretory vessels of hind-body
lcv—lateral collecting excretory vessel
m—mouth
mdv—median dorsal excretory vessel
mpl—posterior lip of fore-body
mv—marginal excretory vessel
mvv—median ventral excretory vessel
oe—oesophagus
omf—outer longitudinal muscle fibers
os—oral sucker
ph—pharynx
plv—primary lateral excretory vessel
pn—posterior nerve
ptcv—posterior transverse commissural excretory vessel
rf—fundaments of reproductive organs
spnc—supra-pharyngeal nerve commissure
vc—vitelline cord
vnw—ventral network of excretory vessels
vv—ventro-lateral excretory vessels of hind-body

Plate XLV

Figs. 1, 2, 4 and 5 were drawn, free-hand, from living specimens, not subject to pressure. Figs. 1, 2 and 3 were not drawn to the same scale.

Fig. 1. *Neascus van-cleavei* (Agersborg) within the cyst.

Fig. 2. *Neascus ambloplitis* Hughes as seen emerging from the cyst.

Fig. 3. *Neascus cuticola* (von Nordmann) within the cyst (after von Nordmann).

Fig. 4. Ventral view of *Neascus van-cleavei* showing the external features of the body and certain internal organs.

Fig. 5. Ventral view of *Neascus van-cleavei* showing the principal vessels of the reserve bladder.

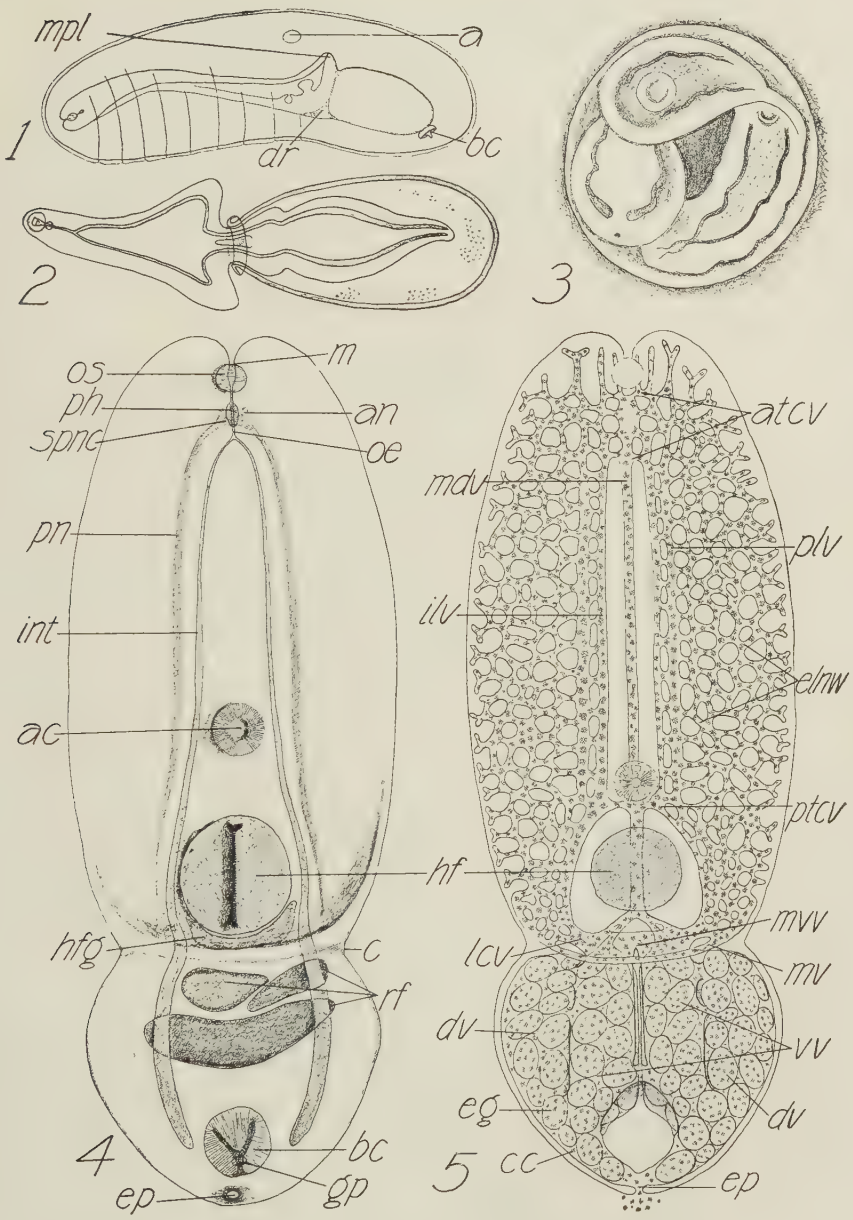
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PLATE XLV

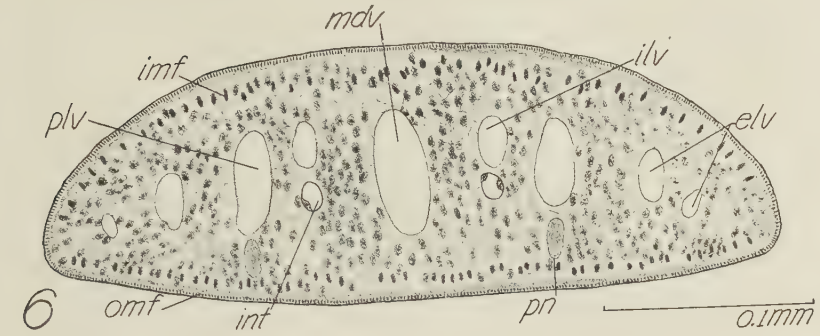
Plate XLVI

Cross-sections of *Neascus van-cleavei* (Agersborg) drawn with the aid of camera lucida.

Fig. 6. Through the region just anterior to the acetabulum.

Fig. 7. Through the hold-fast organ.

Fig. 8. Through the hind-body between the reproductive fundaments and the bursa copulatrix.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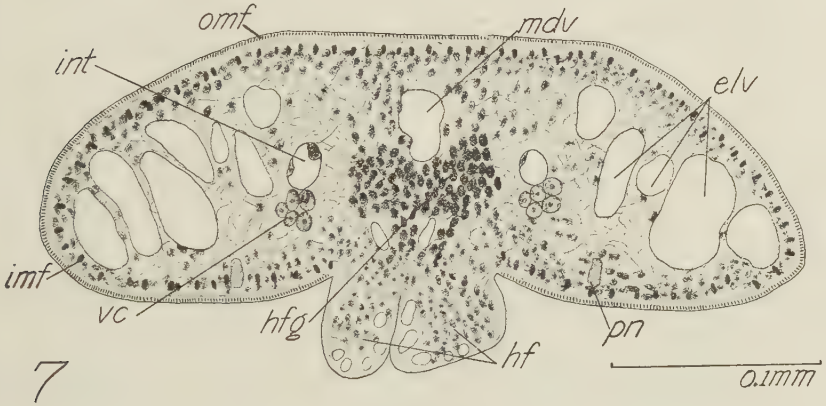
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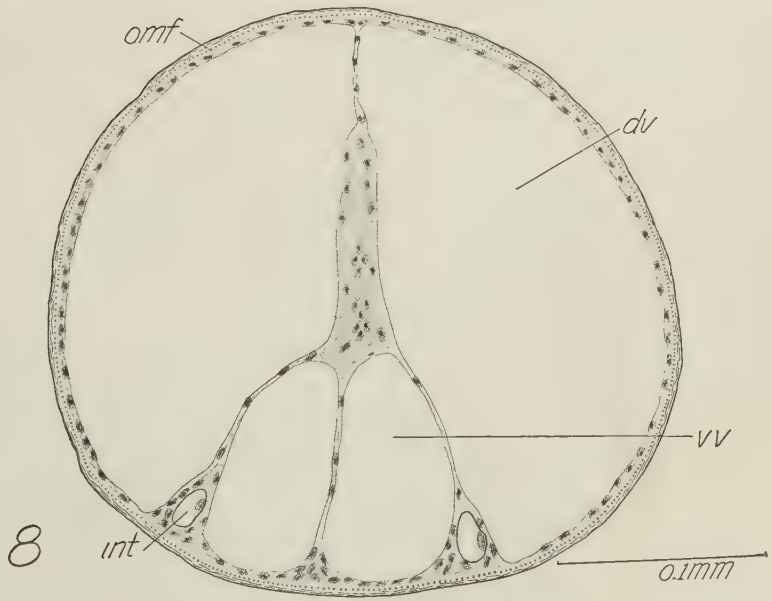
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PLATE XLVI

Plate XLVII

Cross-sections of *Neascus cuticola* (von Nordmann) drawn with the aid of camera lucida.

Fig. 9. Through the hold-fast organ.

Fig. 10. Through the reproductive fundaments.

Fig. 11. Through the hind-body between the reproductive fundaments and the bursa copulatrix.

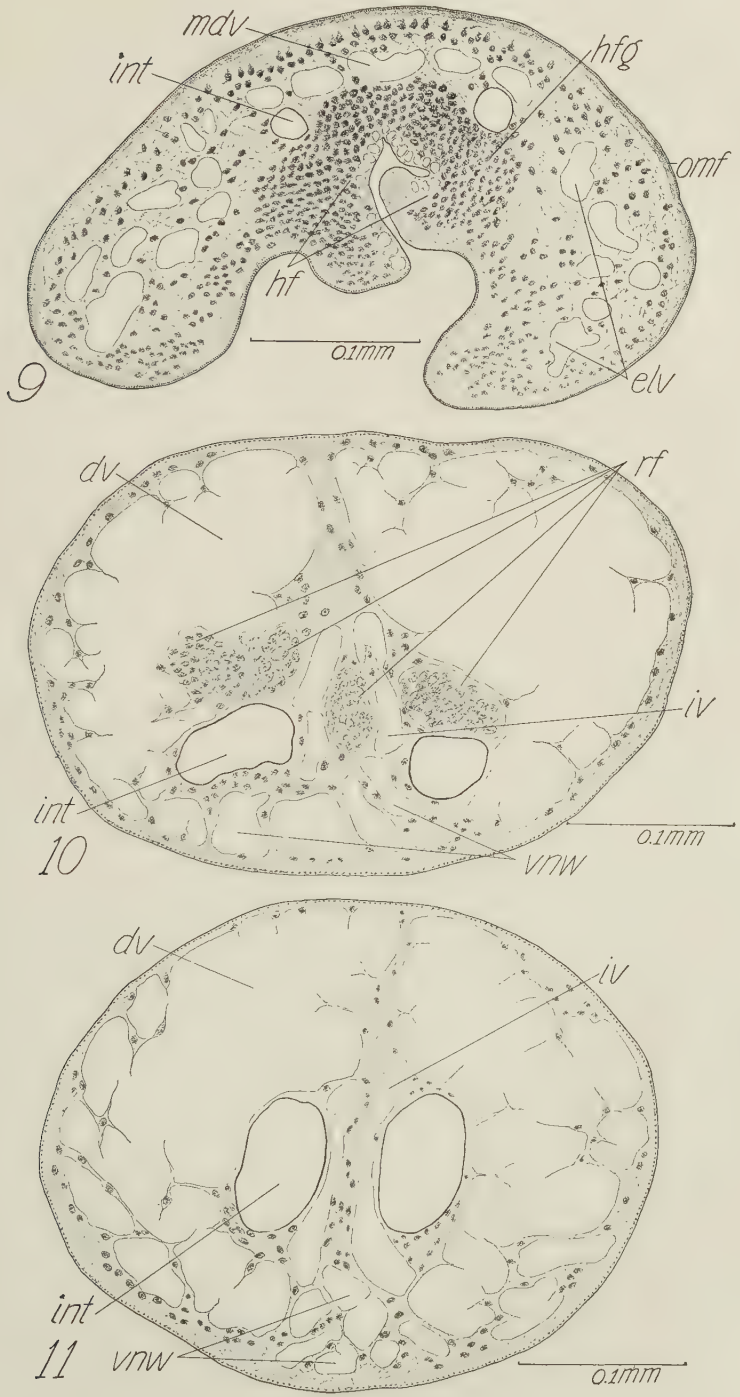
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PLATE XLVII

Studies on the Trematode Family Strigeidae (Holostomidae) No. X *Neascus bulboglossa* (Van Haitsma)

R. CHESTER HUGHES

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. X *NEASCUS BULBOGLOSSA* (VAN HAITSMA) *

R. Chester Hughes
University of Michigan
ANN ARBOR, MICH.

A new species of strigeid metacercaria, *Neascus bulboglossa* (Van Haitsma) determined by an unpublished feeding experiment performed by Mr. Van Haitsma to be the larva of *Crassiphiala bulboglossa* Van Haitsma, was found encysted in *Perca flavescens* Mitchill taken from the mouth of Bessey Creek, a tributary of Douglas Lake, in Cheboygan County, Michigan, during the summer of 1927. The adult trematode is parasitic in the gut of *Ceryle alcyon* Linnaeus in the vicinity of Douglas Lake (Van Haitsma 1925: 122). Although the parasites occur, chiefly, widely distributed in the integument, a few were found in the myotomes and on the gill arches.

Fifteen out of 16 yellow perch, varying in length from 67 mm. to 133 mm. (average 90 mm.), from the mouth of Bessey Creek, were parasitized with from 2 to 530 (average 75) specimens of the present species. The parasite was not found in any of a considerable number of yellow perch collected from other parts of Douglas Lake. A worm, similar to, and perhaps identical with, the present species was briefly described by Cooper (1915: 202; Pl. III, figs. 25, 26) as *Cercaria* sp. from black-pigmented cysts in a minnow.

Most of the observations herein recorded were made on living specimens flattened by cover-glass pressure. For whole mounts, specimens were stained, regressively with a dilute solution of Ehrlich's acid hematoxylin in 70% alcohol. For sectioning, specimens were stained *in toto* in Ehrlich's acid hematoxylin, decolorized on the slide and counterstained with eosin. The present species resembles *Neascus ambloplitis* Hughes more closely than any other species and will be described in comparison with it.

The cyst, like that of *N. ambloplitis* (Hughes 1927: 249) comprises 2 parts, an outer lamellar, syndesmous, black-pigmented portion of host origin and an inner non-cellular thin tough resilient hyalin layer of parasite origin. In 10 specimens, the outer cyst, nearly spherical in shape, varied in diameter from 0.36 mm. to 0.68 mm. (average 0.47 mm). The inner cyst, which closely invests the parasite and can hardly be removed without injury to it, opens readily in a warm bath of com-

* Contribution from the Biological Station and from the Department of Zoölogy, University of Michigan. This is No. X of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

mercial trypsin dissolved in a 0.5% aqueous solution of sodium bicarbonate. As in *N. ambloplitis* the anterior end of the parasite is the first to emerge from the inner cyst. Correlated with the fact that the whole anterior end of the cyst is ruptured this form emerges from the cyst much more readily than *N. ambloplitis.* The inner cyst (Fig. 5), smaller and relatively broader than in *N. ambloplitis,* is somewhat egg-shaped, truncate anteriorly. In *N. ambloplitis* the inner cyst (Fig. 6) is truncate posteriorly. Six inner cysts, containing living parasites and under cover-glass pressure, varied in length from 0.18 mm. to 0.22 mm. (average 0.2 mm.) and in width from 0.14 mm. to 0.18 mm. (average 0.16 mm.).

Although the animal is somewhat folded in the cyst, as is true of *N. ambloplitis,* its principal organs are distinguishable. A constriction (Fig. 2) divides the form of the parasite into well defined fore- and hind-body. Unlike *N. ambloplitis,* the long axis of the freed specimen is not flexed in the region of the constriction (Fig. 3). Freed from the cyst, the parasite is very active; its movements consist of alternately lengthening and shortening the body. Correlated with the absence of an acetabulum, the leech-like movement of *N. ambloplitis* and some other strigeid metacercariae does not occur. In the most extended condition of the body the constriction between the two body regions is smoothed out and the long and slender hind-body is not perceptibly delimited from the swollen fore-body (Fig. 3). In four living specimens, under cover-glass pressure, each in a state of muscular relaxation, the length of the body was 0.34 mm., 0.40 mm., 0.31 mm. and 0.36 mm. respectively. Five mounted specimens varied in length from 0.175 to 0.448 mm. (average 0.27 mm.). *Neascus bulboglossa* is much smaller than *N. ambloplitis.*

The fore-body is deeply cup-shaped. The swollen aspect of the fore-body, even in its most extended condition correlates with the fact that its cup-shaped concavity contains the extraordinarily large hold-fast organ. The surfaces of the fore-body and of the hold-fast organ inside and out are covered quincuncially with minute spines. In four living specimens, under cover-glass pressure, the length of the fore-body was 0.167 mm., 0.182 mm., 0.196 mm. and 0.218 mm. and the width 0.174 mm., 0.17 mm., 0.125 mm. and 0.145 mm. respectively. In five mounted specimens the length of the fore-body varied from 0.112 mm. to 0.193 mm. (average 0.14 mm.) and in width from 0.084 mm. to 0.109 mm. (average 0.096 mm.). In a mounted specimen the thickness of the fore-body was 0.105 mm. In *N. ambloplitis* the fore-body is not so deeply cup-shaped, the ventral lip being relatively much shorter than in the present species.

The hind-body, conical when relaxed, elongated and cylindrical when extended with the pointed posterior end curved retrorsely dorsad (Fig. 3), is less well developed than in *N. ambloplitis.* In four living

specimens under cover-glass pressure it was 0.138 mm., 0.218 mm., 0.16 mm. and 0.145 mm. long and 0.125 mm., 0.087 mm., 0.095 mm. and 0.087 mm. wide respectively. In five mounted specimens the length of the hind-body varied from 0.063 mm. to 0.158 mm. (average 0.128 mm.) and in width from 0.024 mm. to 0.07 mm. (average 0.055 mm.).

The hold-fast organ (Fig. 1, *hf*) is a large bulbous heavily staining structure attached in the usual position by a slender longitudinally elongate pedicle. Its ventral surface is marked by a deep median furrow, the ends of which are shortly bifurcate. In four living specimens under cover-glass pressure the length of the hold-fast organ was 0.111 mm., 0.111 mm., 0.084 mm., and 0.105 mm. respectively.

The relatively large spheroidal oral sucker is ventro-terminal in position. Its diameter in four living specimens under cover-glass pressure, was 0.039 mm., 0.042 mm., 0.039 mm. and 0.045 mm., and in five mounted specimens 0.031 mm., 0.032 mm., 0.035 mm., 0.038 mm., and 0.039 mm. respectively. Ridges on the external surface of the oral sucker such as were described for *N. ambloplitis* (Hughes 1927: 250) were not observed. The relatively large pharynx is a little smaller than the oral sucker. In two living specimens under cover-glass pressure its diameter was 0.021 mm. and 0.024 mm. respectively. According to Van Haitsma (1925: 123) the pharynx in the adult is nearly equal in size to the oral sucker. The very long esophagus bifurcates just in front of the hold-fast organ. The intestinal crura diverge at an obtuse angle about the base of the hold-fast organ, converging a little as they enter the hind-body and extend to the tip of the latter where they terminate blindly (Fig. 2).

Fundaments of reproductive organs, a hold-fast gland and a bursa copulatrix are wanting. The nervous system was not studied. Subcuticular layers of circular and longitudinal muscle fibers are present in both fore- and hind-bodies.

The excretory system of the present species closely resembles that of *N. ambloplitis* (Hughes 1927: 251) and as far as applicable the same terminology is used in the present description. The excretory system of the holostome larva comprises two parts, the primary excretory apparatus consisting of flame cells and associated tubules and the reserve bladder (Faust 1922: 80 and Hughes 1927: 253). As in *N. ambloplitis* the primary excretory apparatus is greatly obscured by the thickness of the animal and the excessive development of the reserve bladder. A few scattered flame cells and tubules were observed but not in sufficient detail to give any definite idea of their arrangement. The excretory system was studied both in living specimens and in sections. The former method was much more fruitful since in preserved material many of the finer details are lost. Sections were, however, indispensable for the study of certain larger features.

The reserve bladder, as in *N. ambloplitis,* comprises an intricate network of large and small vessels, the arrangement of which, although variable in details, shows a high degree of regularity in general plan. These vessels contain a limpid transparent fluid, in which float yellowish, highly refractive, calcareous granules of various sizes. This fluid with the granules contained surges about from one part of the body to another in response to muscular movements and now and then some is discharged from the excretory pore.

The principal vessels of the reserve bladder are as follows: The median dorsal vessel (Fig. 1, *m.*) extends from the region dorsal to the pharynx to about the end of the anterior fourth of the hind-body. In the postero-dorsal part of the hold-fast organ this vessel divides to form a ring about a small island (*hi*). A pair of vessels (*v*) connected with the median dorsal vessel before and behind the hold-fast organ encircle its base. These vessels are connected with the median dorsal vessel by a coarse network of anastomoses (*hn*). Anterior to the hold-fast organ the median dorsal vessel is connected by 4 pairs of transverse commissural vessels (*t*) with a pair of primary lateral vessels (*p*). *Neascus bulboglossa* differs from *N. ambloplitis* in that it has 4 instead of 7 pairs of transverse commissural vessels and that definite intra- and extra-lateral vessels are wanting. The primary lateral vessels are connected with a closed marginal vessel (*me*), which is located in the lip of the cup-shaped concavity of the fore-body, by an irregular network of small anastomoses (*n*). This network is most intensive in the lateral parts of the ventral lip of the fore-body. The primary lateral vessels unite at the juncture of the two body regions to form a short median ventral vessel (*mv*). The arrangement of the vessels in the hind-body is the same as described for the adult by Van Haitsma (1925: 124). The median dorsal vessel bifurcates forming a pair of dorso-lateral vessels (*d*). The median ventral vessel also bifurcates, at a point slightly anterior to the place of division of the median dorsal vessel, forming a pair of ventro-lateral vessels. The ventro- and dorso-lateral vessels on either side anastomose in the posterior third of the hind-body forming a pair of postero-lateral vessels (*pv*). These in turn unite to form a short median excretory duct (*c*) which debouches through the terminal excretory pore (*ep*). Conspicuous semicircular commissures in the hind-body and a muscular excretory bladder are wanting, differing in these respects from *N. ambloplitis.* I was not able to find a connection between the median dorsal and median ventral vessel in the anterior part of the hind-body, such as Van Haitsma (1925: 24) reports for the adult, but such a difference might be due to differences in degree of development. Such a connection occurs in *N. ambloplitis* (Hughes 1927: 253).

DISCUSSION

The present species has all of the characters diagnostic of the larval group Neascus as given by Hughes (1927: 259), having fore- and

hind-bodies well developed and distinctly demarcated, no lateral sucking-cups, fore-body leaf-like, hold-fast organ well developed, reserve bladder highly developed, the smaller branches of which are anastomoses, calcareous granules free in the circumambient fluids, and being encysted. It differs, however, from all other members of the group known to me with respect to (1) the degree of cupping of the fore-body, (2) the large size of the pharynx, (3) the great length of the esophagus, (4) the excessive development of the hold-fast organ, (5) the absence of an acetabulum, (6) the lack of rudiments of reproductive organs and a bursa copulatrix and (7) the peculiar curving of the posterior end of the hind-body, when that part of the body is elongated, a condition which obtains in both living and preserved specimens. It must be acknowledged, however, that in regard to some of these points *Neascus musculicola* (Waldenburg) and *Neascus brevicaudatus* (von Nordmann) have not been carefully studied. In regard to the large size of the pharynx, the excessive development of the hold-fast organ, and the absence of an acetabulum *N. bulboglossa* is unique among the strigeid metacercariae heretofore described. A comparative study of the known species of the larval group Neascus is being presented elsewhere.

I wish here to express my grateful appreciation to Professor George R. La Rue of the Zoölogy Department and Director of the Biological Station, University of Michigan, by whom this study was suggested and under whom it has been conducted. I am also indebted to Mr. J. P. Van Haitsma of the Department of Organic Science, Calvin College, for many helpful suggestions.

Literature Cited

Cooper, A. R. 1915.—Trematodes from marine and fresh-water fishes. Trans. Roy. Soc. Can., (3) 9:181-205, 3 pls.

Faust, E. C. 1922.—Phases in the life history of a holostome *Cyathocotyle orientalis* nov. spec. with notes on the excretory system of the larva. Jour. Parasitol., 8:78-85, 2 pls.

Hughes, R. C. 1927.—Studies on the trematode family Strigeidae (Holostomidae) No. VI. A new metacercaria *Neascus ambloplitis*, sp. nov. representing a new larval group. Trans. Amer. Micr. Soc., 46:248-267, 2 pls.

Van Haitsma, J. P. 1925.—*Crassiphiala bulboglossa*, nov. gen., nov. spec., a holostomatid trematode from the belted kingfisher, *Ceryle alcyon* Linn. Trans. Amer. Micr. Soc., 44:121-131, 2 pls.

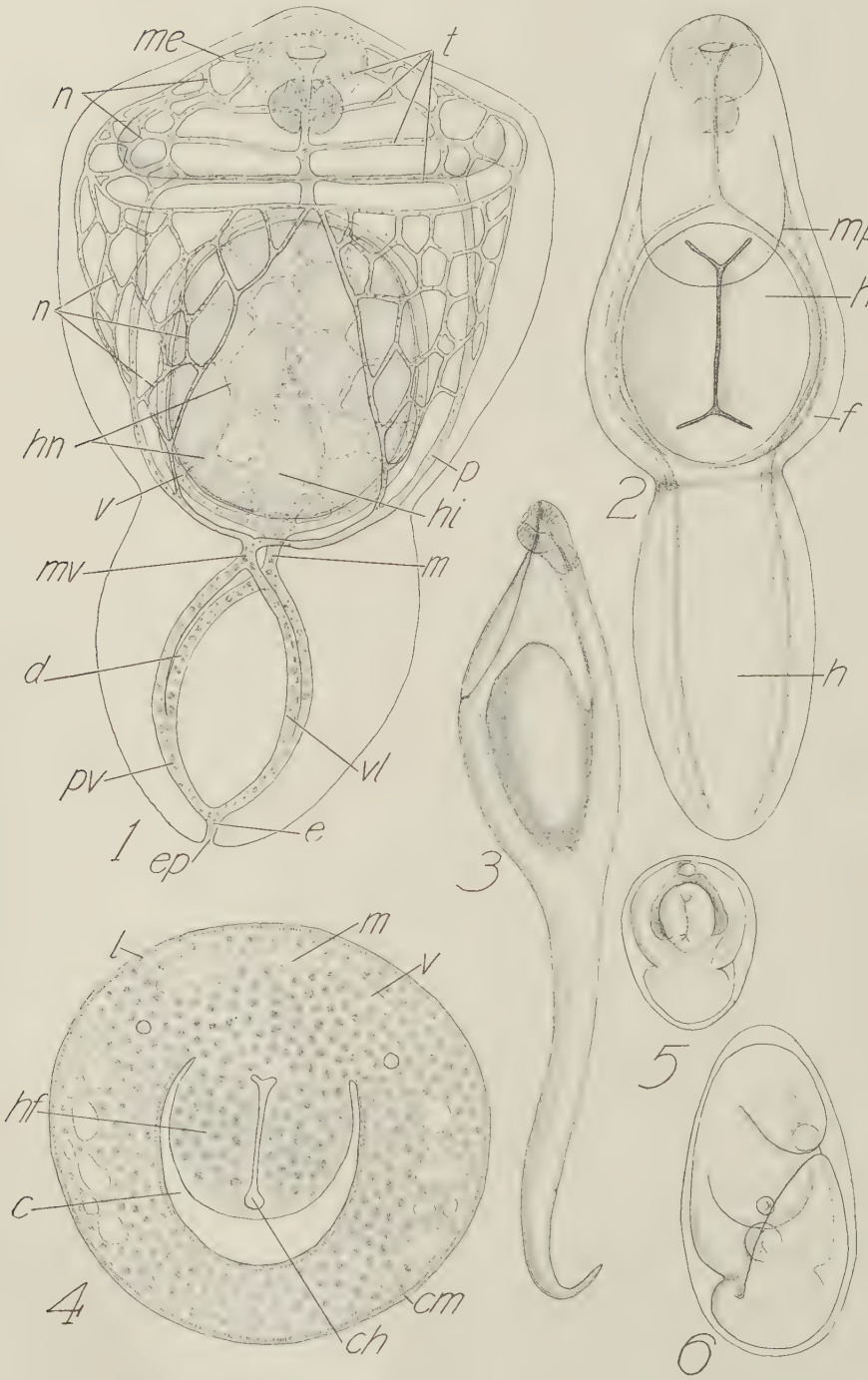

PLATE V

Abbreviations Used

c—concavity of fore-body
ch—cavity of hold-fast organ
cm—circular muscle fibers
d—dorso-lateral excretory vessel
e—excretory duct
ep—excretory pore
f—fore-body
h—hind-body
hf—hold-fast organ
hi—island in median dorsal excretory vessel in base of hold-fast organ
hn—network of excretory vessels in base of hold-fast organ
l—longitudinal muscle fibers
m—median dorsal excretory vessel
mp—margin of posterior lip of fore-body
me—marginal excretory vessel
mv—median ventral excretory vessel
n—network of small excretory vessels connecting primary lateral excretory vessel with marginal excretory vessel
p—primary lateral excretory vessel
pv—postero-lateral excretory vessel
t—transverse commissural excretory vessels
v—excretory vessel encircling base of hold-fast organ
vl—ventro-lateral excretory vessel

Explanation of Plate V

Figs. 1, 2, 3, 5 and 6 were drawn, free-hand, from living specimens. Figs. 5 and 6 were drawn to the same scale. Figs. 1, 2 and 3 were drawn to different scales and represent different states of contraction. Except Fig. 6 all represent *Neascus bulboglossa.*

Fig. 1.—Ventral view in much shortened condition, showing principal vessels of so-called reserve bladder.

Fig. 2.—Ventral view in state of muscular relaxation, showing digestive system and external features of body.

Fig. 3.—Side view in much elongated condition showing peculiar cup-shaped conformation of fore-body and large hold-fast organ.

Fig. 4.—Cross-section through region of hold-fast organ. × 621.5 approx.

Fig. 5.—Inner cyst.

Fig. 6.—Inner cyst of *Neascus ambloplitis* drawn to the same scale as Fig. 5.

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE). NO. XII. *AGAMODISTOMUM LA-RUEI* SP. NOV.[1]

By R. CHESTER HUGHES.
University of Michigan, Ann Arbor, Michigan, U.S.A.

(With 3 Figures.)

Twelve larval trematodes, herein described as *Agamodistomum la-ruei* n.sp., were found by Prof. La Rue, at the autopsy of a racoon, *Procyon lotor lotor* (Linn.), which had been kept for several months in captivity near Douglas Lake, Cheboygan County, Michigan, during the spring and summer of 1925. The parasites were found by teasing the lungs apart in water. It was not determined whether they were in the alveoli of the lungs or in the tissue. They were apparently not encysted.

The specimens, fixed in sublimate acetic and stained in a dilute solution of Ehrlich's acid haematoxylin in 70 per cent. alcohol, were mounted ten *in toto* on one slide, and two in sections of 8 μ thickness on another[2]. The sections were counterstained with eosin. Living specimens were not studied.

Agamodistomum la-ruei closely resembles *A. marcianae*, which is parasitic in the garter snake, *Thamnophis marciana* (Baird and Girard), according to La Rue (1917), and in frogs, *Rana pipiens* Schreber and *R. clamitans* Latreille, according to Cort (1918). *A. la-ruei*, however, is somewhat smaller than *A. marcianae*, and since it occurs in a homothermous host it is probable that it belongs to a distinct species. Because of its similarity to *A. marcianae* as described by La Rue (1917, p. 3) it is unnecessary to give a detailed morphological description of *A. la-ruei*. The principal measurements of the ten specimens which were mounted *in toto* are shown in the accompanying table. A comparison of these measurements with those of *A. marcianae* (La Rue, 1917, p. 6) which are also of preserved specimens mounted *in toto* shows that the present species is consistently smaller. The salient features of *A. la-ruei* are shown in Figs. 1 and 2.

A comparison with type and cotype specimens (mounted *in toto* and in sections) of *A. marcianae*, which were kindly given to me for examination by

[1] Contribution from the Biological Station and the Zoological Laboratory, University of Michigan. This is the twelfth of a series of studies on the family Strigeidae by Prof. George R. La Rue, his students and associates.

[2] These slides bear Catalogue Nos. 278 and 279 in the parasite collection, Museum of Zoology, University of Michigan.

Prof. La Rue, shows that in general *A. la-ruei* differs as follows. The anterior end of the body is seldom constricted at the base of the oral sucker as it frequently is in *A. marcianae*, and the ducts of the penetration glands are somewhat larger in diameter and less coiled. These ducts are in general more sinuous, however, than Fig. 1 indicates.

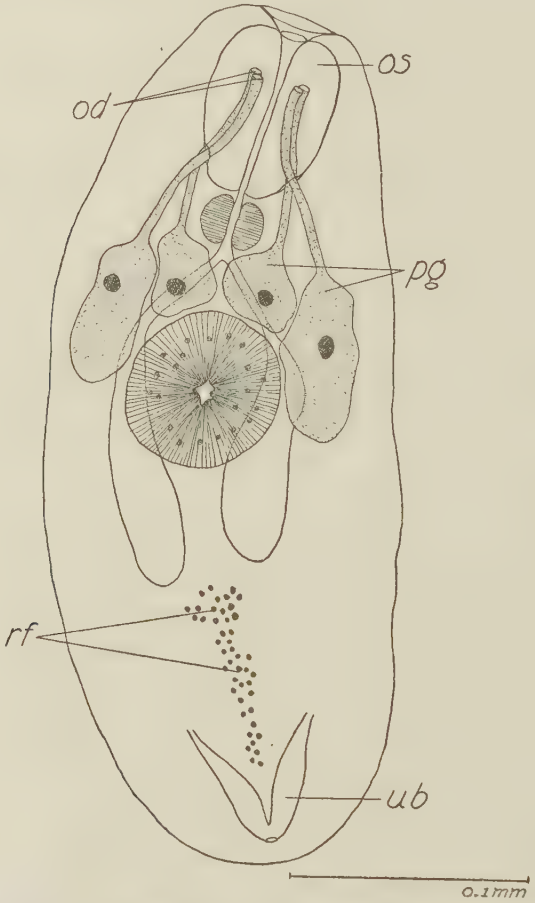

Fig. 1. *Agamodistomum la-ruei*, ventral view of a preserved specimen mounted *in toto*, drawn with the aid of a camera lucida. *od*, external openings of the ducts of the penetration glands; *os*, oral sucker; *pg*, penetration glands; *rf*, fundament of reproductive organs; *ub*, urinary bladder.

Measurements in Millimetres of Ten Preserved Specimens of Agamodistomum la-ruei *mounted* in toto.

		Minimum	Maximum	Average
Body	Length	0·275	0·353	0·307
	Width	0·148	0·190	0·164
Oral sucker	Length	0·056	0·081	0·069
	Width	0·045	0·056	0·051
Acetabulum	Length	0·056	0·070	0·064
	Width	0·063	0·074	0·068

Discussion.

It has been shown by Cort (1918) and Cort and Brooks (1928, p. 199) that *Agamodistomum marcianae* develops from a strigeid cercaria. The larva, however, is hardly comparable to other strigeid metacercariae since after penetra-

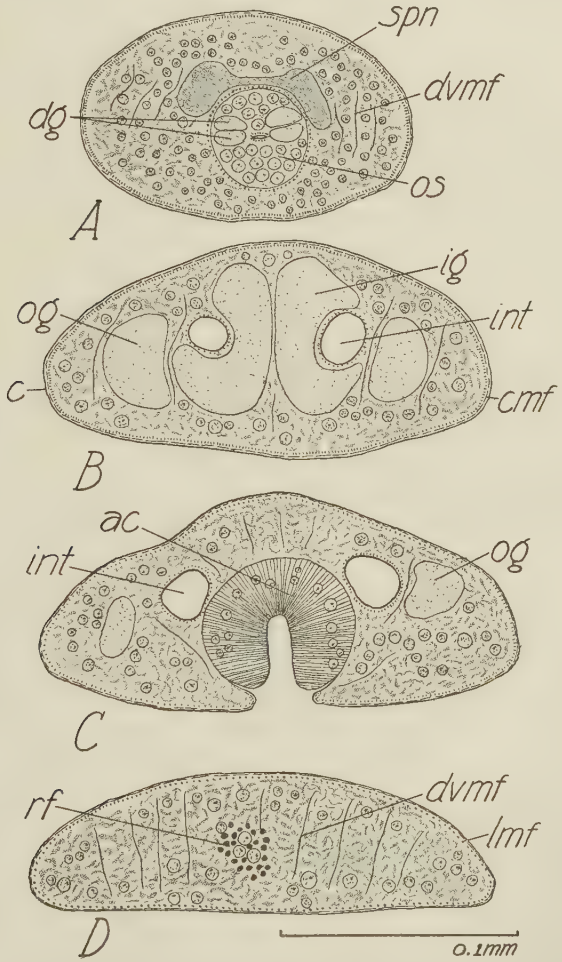

Fig. 2. *Agamodistomum la-ruei*, cross-sections drawn with the aid of a camera lucida: *A*, through oral sucker; *B*, between oral sucker and acetabulum; *C*, through acetabulum; *D*, through fundament of reproductive organs: *ac*, acetabulum; *c*, cuticle; *cmf*, circular muscle fibres; *dg*, ducts of penetration glands; *dvmf*, dorso-ventral muscle fibre; *ig*, inner penetration gland; *int*, intestinal caecum; *lmf*, longitudinal muscle fibres; *og*, outer penetration gland; *os*, oral sucker; *rf*, fundament of reproductive organs; *spn*, supra-pharyngeal nerve commissure.

tion into the second intermediate host it regenerates the penetration glands and, although it grows to much greater size, fails to undergo the profound metamorphosis common to most strigeids. These authors suggest that this agamodistome represents either an aberrant development brought about by

the penetration of the cercaria into an unsuitable host in which it is able to maintain itself and grow but not metamorphose, or the intercalation of an additional phase, involving an additional host, into the usual strigeid life cycle. The close similarity of *A. la-ruei* to *A. marcianae* suggests that it, too, may belong to the family Strigeidae. Descriptions and reports of other agamodistomes of similar structure occur in the literature. In fact not enough is known of these forms to preclude the possibility of general conspecificity. In the following synopsis these forms are all brought together on the supposition that they may all be strigeids. The parasites included in this synopsis are agamodistomes having in common a similar body form and four large penetration glands situated about the acetabulum. They differ from typical strigeid metacercariae in the complete absence of hind-body, hold-fast organ and reserve bladder.

AGAMODISTOMES SIMILAR TO *A. LA-RUEI* IN POIKILOTHERMOUS HOSTS.

1. **Agamodistomum tetracystis** (Gastaldi) mihi.

SYNONYMY: *Distoma tetracystis* Gastaldi (1854, p. 4).
Distomum tetracystis Ranae esculentae Gastaldi Diesing (1855 *a*, p. 64).
Distomum Tetracystis Gastaldi Diesing (1855 *b*, p. 390).
Distomum tetracystis Gast. Braun (1893, p. 871).

This form, parasitic in *Rana esculenta* in Europe, was briefly described by Gastaldi (1854, p. 4, Pl. 1, figs. 1–3). A description based on that of Gastaldi was published by Stossich (1889, p. 68). According to the figures and descriptions *A. tetracystis* differs from *A. la-ruei* and *A. marcianae* in that the oesophagus is long and slender and the pharynx is somewhat removed from the oral sucker. De Filippi (1855, p. 339) called attention to a strong similarity of *A. tetracystis* to *Cercaria microcotyla* de Filippi. Following this suggestion of de Filippi, Diesing (1855 *b*, p. 390) considered the two forms to be genetically related.

2. **Agamodistomum marcianae** (La Rue) Cort.

SYNONYMY: *Cercaria marcianae La Rue* La Rue (1917, p. 3).

This form was described in detail from preserved material by La Rue (1917, pp. 3–8, Pl. 1, figs. 1, 2). The spination and excretory system were studied in more detail in living specimens by Cort (1918, figs. 1, 2, 3), who also demonstrated that it was possible to introduce *A. marcianae* into garter snakes, *Thamnophis sirtalis sirtalis* (Linn.), by feeding the snakes with infected tadpoles. In this change of hosts the parasite shows no advance in development. Cort and Brooks (1928, p. 199) showed that *A. marcianae* develops from a strigeid cercaria, *Cercaria marcianae* (La Rue). *A. marcianae* has been found in *Thamnophis marciana* (Baird and Girard), *T. s. sirtalis* (Linn.), *Rana pipiens* Schreber and *R. clamitans* Latreille.

Notes and sketches pertaining to the distribution of *A. marcianae* in heavily infected tadpoles, prepared under the direction of Prof. La Rue by Mr Thomas Blair have been kindly given to me. These tadpoles had been

experimentally infected by Prof. W. W. Cort and Mr S. T. Brooks at Douglas Lake during the summer of 1925 and sent to Mr Blair in Ann Arbor where the following observations were made. The worms occur, free, chiefly in the subcutaneous lymph spaces and are most abundant in the ventral part of the abdomen (Fig. 3). A few were found in the deeper tissues. The specimens shown on the eyes (Fig. 3) were situated just beneath the skin covering the eye.

Fig. 3. A tadpole, *Rana pipiens* Schreber, heavily infested with *Agamodistomum marcianae* (La Rue). Four views (ventral, sinistral, dorsal, and dextral) showing the distribution of the parasites, chiefly in the subcutaneous lymph spaces. This tadpole contained (by count) 587 agamodistomes. Redrawn from sketches made with the aid of a camera lucida by Mr Thomas Blair.

3. **Agamodistomum ordinata** (Nicoll) mihi.

Synonymy: *Cercaria ordinata* Nicoll (1912, p. 767).

This species was described by Nicoll (1912, p. 767, text-fig. 107 A) from living specimens taken from three North American striped snakes which died in the Gardens of the Zoological Society of London. The conditions under which these snakes had been kept were not recorded and it is not possible to

determine whether they acquired their infections in England or in America. The agamodistome compares favourably in size and structure with *Agamodistomum marcianae* except that according to the figure the two penetration glands on either side pour their secretions into a common duct, whereas in *A. marcianae* each gland has a separate duct. These apparent differences may be due to an error in observation and it is possible that the two species are identical.

In Homothermous Hosts.

4. **Agamodistomum putorii** (Molin) Stossich.

Synonymy: *Distomum putorii* Molin (Molin 1858, p. 131).
Distomum putorii Molin (Braun 1893, p. 870).

Briefly described by Molin (1861, p. 224, Pl. V, fig. 4) this parasite was better described by von Linstow (1875, p. 192, Pl. II, fig. 5). A brief, but not original, description was published by Stossich (1892, p. 34). The parasite was found encysted in the muscles and on the oesophagus of *Foetorius putorius*. The similarity of this species with *Agamodistomum tetracystis* was observed by von Linstow. According to von Linstow's measurements *A. putorii* is considerably larger than *A. la-ruei*.

5. **Agamodistomum suis** Stiles.

Synonymy: *Distomeen in Schweinefleisch* Duncker (1881 *a*, p. 23)
Muskel-Distomeen Duncker (1881 *d*, p. 141).
Distomum sp. *Duncker* Braun (1893, p. 870).
Distomum musculorum suis Duncker (1896, p. 279).
Agamodistomum suis Stiles (1898, p. 28).
Leberegel aus dem Schweinefleisch Kästenbaum (1899, p. 244).

This parasite was first described and figured by Duncker (1881 *a*, p. 23, figs. 1–6) from specimens found by G. Leunis, a trichina inspector in Saxony. Other descriptions based on the original by Duncker have been published by Duncker (1881 *d*, p. 159, fig. 1; 1884, p. 39, figs. 1–6 and 1896, p. 278, figs. 1–8), Leuckart (1889, p. 154, fig. 86) and Stiles (1898, p. 28, fig. 1) and a figure by Kästenbaum (1899, fig. 33). Its occurrence has also been reported by Duncker (1881 *b*, p. 55; 1881 *c*, p. 154 and 1881 *c*, p. 141), Leuckart (1881, p. 46) and Schneidemuehl (1896, p. 302). The specimens reported from this country by Stiles (1900, p. 559) were not definitely identified with the present species. *Agamodistomum suis* is rare, being found only occasionally, encysted in the muscles of swine, *Sus scrofa domestica*, by European meat inspectors. According to measurements given by Stiles (1898) *Agamodistomum suis* is somewhat larger than *A. la-ruei*. According to the figure of Leuckart it differs from *A. la-ruei* in that the oral sucker is relatively shorter and the reproductive system is represented by three distinct fundaments.

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I wish here to express my grateful appreciation to Prof. George R. La Rue of the Zoological Laboratory and Director of the Biological Station, University of Michigan, by whom this work was suggested and under whom it has been conducted.

I am also indebted to Prof. La Rue for the use of the type specimens of *Agamodistomum la-ruei* and type and cotype specimens of *A. marcianae* all of which belong to his personal collection. My thanks are due to Mr Thomas Blair, of the Medical School of the University of Michigan, for the use of notes and sketches relative to the distribution of *Agamodistomum la-ruei* in tadpoles which had been experimentally infected by Prof. W. W. Cort and Mr S. T. Brooks.

REFERENCES.

Braun, M. (1893). Vermes. In *Bronn's Klassen und Ordnungen des Thier-Reichs.* **4**, Abt. 1 A, Lief. 28–30, pp. 817–925. Leipzig.

Cort, W. W. (1918). The excretory system of *Agamodistomum marcianae* (La Rue), the agamodistome stage of a forked-tailed cercaria. *J. Parasitol.* **4**, 130–134, 3 figs.

Cort, W. W. and Brooks, S. T. (1928). Studies on the holostome cercariae from Douglas Lake, Michigan. *Trans. Amer. Microsc. Soc.* **47**, 179–221, 8 text-figs, 5 pls.

Diesing, K. M. (1855 *a*). Neunzehn Arten von Trematoden. *Denkschr. d. k. Akad. d. Wiss.* Wien. Math.-nat. Kl. **10** (1. Abt.), 59–70, 3 pls.

—— (1855 *b*). Revision der Cercarieen. *Sitzungsb. d. k. Akad. d. Wiss.* Wien. Math.-nat. Kl. **15**, 377–400.

—— (1858 *a*). Berichtigungen und Zusätze zur Revision der Cercarieen. *Ibid.* **31**, 239–290.

—— (1858 *b*). Revision der Myzhelminthen. Abtheilung Trematoden. *Ibid.* **32**, 307–390, 2 pls.

Duncker, H. C. J. (1881 *a*). Distomeen im Schweinefleisch. *Zeitschr. f. mikr. Fleischschau,* Berlin, **2**, 23–25, figs. 1–6.

—— (1881 *b*). *Ibid.* **2**, 55.

—— (1881 *c*). *Ibid.* **2**, 154.

—— (1881 *d*). *Ibid.* **2**, 159–160, 1 fig.

—— (1881 *e*). Muskel-Distomeen. *Ibid.* **2**, 141.

—— (1884). Distomeen im Schweinefleische. *Zeitschr. f. mikr. Fleischschau,* **3**, 39–42, 6 figs.

—— (1896). Die Muskeldistomeen. *Berlin. thierärztl. Wochenschr.* **24**, 279–282, 8 figs.

de Filippi, F. (1855). Mémoire pour servir à l'histoire génétique des trématodes. *Mem. d. R. Accad. d. Sc. Torino* (2), **15** (Cl. d. Sc. Fis. e Matt.), 331–358, 2 pls.

Gastaldi, B. (1854). *Cenni sopra alcuni nuovi elminti della Rana esculenta, con nuove osservazione sul Codonocephalus mutabilis* Diesing, 14 pp. Torino.

Kästenbaum, H. (1899). *Grundriss der Thierseuchen und Parasiten-Krankheiten.* 281 pp. Wien und Leipzig.

La Rue, G. R. (1917). Two new larval trematodes from *Thamnophis marciana* and *Thamnophis eques. Occasional Papers Mus. Zool. Univ. of Mich.* No. 35, 14 pp. 1 pl.

Leuckart, K. F. R. (1881). Distomeen in Schweinefleisch. *Zeitschr. f. mikr. Fleischschau,* Berlin, **2**, 46.

—— (1889). *Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten.* 2. Aufl. Vol. **1** (2. Abt.), 897 pp. Leipzig.

von Linstow, O. F. B. (1875). Beobachtungen an neuen und bekannten Helminthen. *Arch. f. Naturg.* **41**, 183–207, 3 pls.

Molin, R. (1858). Prospectus helminthum, quae in prodromo faunae Venetiae continentur. *Sitzungsb. d. k. Akad. d. Wiss.* Wien, Math.-nat. Kl. **30**, 127–158.

—— (1861). Prodromus faunae helminthologicae Venetae. *Denkschr. d. k. Akad. d. Wiss.* Wien, Math.-nat. Kl. **19** (2. Abt.), 189–338, 15 pls.

Nicoll, W. (1912). On two new larval trematodes from the striped snake (*Tropidonotus ordinatus sirtalis*). *Proc. Zool. Soc. London* (1912), 767–770, 1 fig.

Schneidemuehl, G. (1896). *Lehrbuch der vergleichenden Pathologie und Therapie des Menschen und der Hausthiere*, etc. 2. Lief., pp. 209–448. Leipzig.

Stiles, C. W. (1898). The flukes and tapeworms of cattle, sheep, and swine with special reference to the inspection of meats. *Bureau of Animal Industry, U.S. Department of Agriculture, Bulletin* No. 19, pp. 11–136, 124 figs.

—— (1900). Notes on Parasites. *U.S. Bureau of Animal Industry*, 16*th Ann. Report*, pp. 558–611, 7 text-figs, 2 pls.

Stossich, M. (1889). I distomi degli anfibi. *Boll. Soc. adriat. di sc. nat. Trieste*, **11**, 60–74.

—— (1892). I distomi dei mammiferi. *Programma d. civica Scuola Reale Superiore*, Trieste, 42 pp.

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (Holostomidae) NO. XIII. THREE SPECIES OF TETRACOTYLE

R. CHESTER HUGHES

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. XIII. THREE NEW SPECIES OF TETRACOTYLE.*

R. Chester Hughes
University of Michigan, Ann Arbor, Mich.

During the spring (March and April) of 1927 tetracotyliform larvae of apparently three different species were found encysted on the heart and in the pericardial cavity of fishes. Three species of Tetracotyle from fishes in Europe and one in Turkestan have been described but none, heretofore have been reported from fishes in this country. The name Tetracotyle is here used in its restricted sense, namely, to include only those strigeid metacercariae having lateral cotylae, a slightly developed hind-body and a *reserve bladder* consisting of "an irregular continuous coarse-meshed network of spaces, not in the form of definite vessels" (Hughes 1927: 257, 260). Ashworth and Bannerman (1926: 167) regarded the presence, on either side of the oral sucker, of a lateral organ capable of suctorial action as a character unique to the group Tetracotyle, even in its restricted application. In my opinion the morphology of the reserve bladder is a character of greater taxonomic importance than the presence or absence of lateral suckers. In a previous paper (Hughes 1927: 257) I pointed out that in *Diplostomum volvens* von Nordmann, of which the excretory system was studied in detail by Claparède (1858: 97) and Fraipont (1880:430), the reserve bladder consists of small, distinct, regularly disposed vessels with calcareous corpuscles confined to vesicles at the termini of small branches. Thus *Diplostomum petromyzi-fluviatilis* Diesing and *Tetracotyle phoxini* Faust are not to be classed as Tetracotyles since in them the reserve bladder, according to Brown (1899) for the one and Mataré (1910) and Ashworth and Bannerman (1927) for the other, is essentially like that of *Diplostomum volvens*.

Method of Study. The species described in this paper were studied both alive and in preserved material fixed with sublimate-acetic. For whole mounts specimens were stained regressively in Ehrlich's acid haematoxylin. For sections (10μ) specimens were stained *in toto* in Ehrlich's acid haematoxylin, destained on the slide and counterstained with eosin. For purposes of comparison I have made use of several specimens of Tetracotyle from the body-cavity of *Phoxinus sp.* from the vicinity of Lunz, which were presented to Professor La Rue by Professor L. Scheuring of München. These

* Contribution from the Biological Station and from the Department of Zoölogy, University of Michigan. This is the thirteenth of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

specimens were stained as outlined above, some mounted *in toto* and some sectioned. These are apparently identical with *Tetracotyle variegata* (Creplin) mihi. I have studied in the same manner several cotype specimens of *Tetracotyle sogdiana* Pavlovsky and Anitchkov which were presented to Professor La Rue by Professor E. N. Pavlovaky of Leningard and specimens of *Tetracotyle percae-fluviatilis* von Linstow, which were presented to Dr. La Rue by Dr. Ferdinand Zandt of Konstanz. All of the measurements of living specimens were taken from parasites subjected to cover-glass pressure. Care was taken, however, to keep plenty of water under the cover-glass so that the pressure would be as nearly constant and as little as possible.

Tetracotyle communis sp. nov.*

The type specimens of this species were taken from 8 adult *Stizostedion canadense griseum* (De Kay) caught in Lake Erie in the latter part of March 1927. On the average over 100 specimens were taken from each of these fishes in which they were found only on and about the heart. Similar worms, apparently morphologically identical with the type specimens, were found in great abundance in *Percopsis omisco-maycus* (Walbaum) and less abundantly in *Catostomus commersonnii* (Lacépède) from Douglas Lake, Michigan, during the summer of 1927. In these latter fishes the parasites were sometimes also found encysted in the connective tissues of the eyeball and in the adipose tissue of the eye-socket. In the pericardial cavity the grape-like clumps of cysts generally occur in masses of adipose tissue.

The Cyst. The cyst, nearly spherical in shape, comprises two parts, an outer syndesmous lamellar portion of host origin and an inner delicate hyalin layer of parasite origin which closely invests the worm (fig. 1). The cysts are easily opened at any time without injury to the parasites, but some collected in the latter part of April were so delicate that they could hardly be removed from the fish without being ruptured. The delicacy of the cyst in the late spring may be due to the digestive action of an enzyme secreted by the parasite itself and is probably associated with the rise in temperature of the water in which the host lives. The wall of the cyst varies in thickness from 0.15 mm. to 0.23 mm. Ten cysts, containing living parasites and not subjected to pressure, varied in diameter from 0.98 mm. to 1.33 mm. (average 1.09 mm.).

The Parasite. Within the cyst (fig. 1) the parasite is surrounded by a small quantity of sticky gelatinous fluid containing excretory granules. Removed from the cyst the parasite is very active. Those collected in April were especially active. These specimens, placed in water in a watch glass crawled in the manner of a leech, using the oral sucker and acetabu-

* Mounted type and paratype specimens of *T. communis* bear Catalog Nos. 252 to 258 in the Parasite Collection, Museum of Zoology, University of Michigan.

lum as organs of attachments, on the surfaces of the container and on the under side of the surface film of the water. They also showed a tendency to cling to the needles with which they had been removed from the cysts and to crawl up the needles out of the water.

The specimens collected in March were exceedingly tenacious of life. The fishes from which they were taken had been dead and kept on ice for several days before the cysts were removed and placed in distilled water. The parasites in these cysts, kept at room temperature, lived for 13 days when they were allowed to die of dessication. It was possible to keep specimens alive for several hours under a cover-glass and under considerable pressure provided they were not allowed to dry.

In dorsal or ventral view, the paraite is ovate. In side view (fig. 5) it is thick-bodied with the dorsum gently arched and the ventrum slightly concave. The acetabulum and hold-fast organ are visible as low median prominences in the posterior half of the ventral concavity, and the hind-body as a low median elevation on the posterior end of the dorsum (fig. 5). The margins of the animal are thick, not foliaceous as in many strigeid metacercariae. The entire surface of the body is spinous. Mucous extrusions from subcuticular glands were not observed. The concavity of the ventral surface quickly disappears in specimens placed in water or in fixative. Five living specimens under cover-glass pressure varied in length from 0.57 mm. to 0.95 mm. (average 0.8 mm.) and in width from 0.57 mm. to 0.9 mm. (average 0.7 mm.). Ten mounted specimens varied in length from 0.51 mm. to 0.75 mm. (average 0.62 mm.) and in width from 0.42 mm. to 0.6 mm. (average 0.53mm.).

The hold-fast organ (figs. 5, 6, 10, *hf*) consists of a transversely elongate mass of heavily staining cells contiguous with the ventral body-wall near the posterior end of the body. Its relatively simple cavity (fig. 6, *chf*) communicates with the exterior through a small circular opening (fig. 6, *ohf*). The inner-most recesses of this cavity are not visible in living specimens. In mounted specimens the cavity is transversely elongate with unbranched antero- and postero-lateral ramifications. The spinous inner surfaces of the hold-fast organ were never seen to be everted. The hold-fast organ is suspended from the dorsal body-wall by a pair of short longitudinal *hold-fast mesenteries* (fig. 10, *hfm*) attached to its dorso-lateral margins. In 5 living specimens under cover-glass pressure the hold-fast organ varied in length from 0.14 mm. to 0.2 mm. (average 0.17 mm.) and in width from 0.26 mm. to 0.36 mm. (average 0.3 mm.). In 6 mounted specimens the length varied from 0.08 mm. to 0.14 mm. (average 0.12 mm.) and the width from 0.14 mm. to 0.18 mm. (average 0.16 mm.). In each of two living specimens the diameter of the external opening of the hold-fast organ measured 0.03mm. in diameter. The tissues of the hold-fast organ are glandular but a distinct separate hold-fast gland is not present.

The well developed, transversely elongate, acetabulum (figs. 5, 6, 9, *ac*), is located immediately in front of the hold-fast organ in the anterior part of the second half of the body. In 5 living specimens under cover-glass pressure, it varied from 0.14 mm. to 0.2 mm. (average 0.17 mm.) in length and from 0.18 mm. to 0.22 mm. (average 0.2 mm.) in width. In 10 mounted specimens it varied from 0.11 mm. to 0.16 mm. (average 0.13 mm.) in length and from 0.15 mm. to 0.18 mm. (average 0.17 mm.) in width. In preserved specimens the acetabulum is longer than the hold-fast organ and is of about the same width.

The lateral suckers (figs. 6, 7, *ls*) are located on the ventral surface on either side of and a little back of the oral sucker and are distinctly smaller than that organ. They consist of inpocketings of the ventral cuticula; the parenchymal tissue contiguous with the body-wall at these places being somewhat more extensive and thicker than elsewhere. These masses of parenchymal tissue do not constitute conspicuous "antero-lateral prominences" such as were described for *Tetracotyle pipientis* Faust (Hughes 1928: 44). In living specimens the cavity of the lateral sucker is usually ovoidal with a narrowed mouth of variable shape. The lateral suckers in this species are apparently never everted. In fixed material the cavity of the sucker is dagger-shaped, the blade representing a longitudinal cavity canopied by the ventral body wall and the hilt an anterior external opening (fig. 11). In 3 living specimens under cover-glass pressure the length of the cavities of the lateral suckers were 0.09 mm., 0.075 mm. and 0.075 mm. The mouths of these cavities were 0.045 mm., 0.045 mm. and 0.03 mm. respectively, in width. The length of the cavities in 5 mounted specimens varied from 0.052 mm. to 0.105 mm. (average 0.081 mm.). A large number of unicellular glands (figs. 7, 8, *cg*) are apparently associated with the lateral suckers. Similar glands were found by Pavlovsky and Anitchkov (1922: 310; Pl. XXV, fig. 6) in *Tetracotyle sogdiana*, and were interpreted to be cystogenous.

The spheroidal, ventro-terminal, oral sucker (fig. 6, *os*), in 5 living specimens under cover-glass pressure, varied in length from 0.12 mm. to 0.15 mm. (average 0.133 mm.) and in 10 mounted specimens from 0.087 mm. to 0.133 mm. (average 0.117 mm.). The posterior half of the oral sucker contains many large unicellular glands. The pharynx (figs. 6, 7, *ph*), small and apparently poorly developed, is difficult to see in both living specimens and whole mounts. It measured 0.036 mm. long in each of two living specimens under cover-glass pressure. The oesophagus (fig. 6 *oe*) is short and narrow. The caecal furcae (figs. 5, 6, 8–10, *int*) are broader than the oesophagus and extend dorso-lateral to the acetabulum and hold-fast organ, terminating blindly just back of the latter.

The nervous system comprises a supra-pharyngeal commissure (fig. 6 *spnc*) and two pairs of nerve trunks, one pair extending anteriorly (*an*)

and the other posteriorly (*pn*) from the ends of the commissure. The anterior trunks immediately bifurcate giving rise to two pairs of nerves, one of which goes to the regions lateral to the oral sucker and the other gives rise to two pairs of branches to the anterior parts of the lateral suckers. The posterior trunks give off a pair of branches to the posterior parts of the lateral suckers and a pair to the anterior part of the hold-fast organ, and were traced back to the hinder part of the hold-fast organ whence they could not be followed further. All of the larger nerves lie near the ventral body-wall.

The musculature consists of dorso-ventral fibers (figs. 8, 9, *dvmf*) and subcuticular layers of circular and longitudinal fibers (figs. 7, 10, *cmf*, *lmf*).

The reproductive system is represented by a single small heavily staining mass of cells, located in the anterior part of the median septum of the hind-body. No traces of a bursa copulatrix or genital pore were found.

The excretory system of the present species is similar to those described by Faust (1922: 80) for *Tetracotyle orientalis* Faust and by Hughes (1928: 48) for *Tetracotyle pipientis* Faust. It consists of two parts, a *primary excretory apparatus* comprising flame-cells and associated tubules, and a *reserve bladder*.

The study of the solenoctye pattern, which can be done only in living material because all traces of the smaller vessels are lost in preservation, is rendered exceedingly difficult by the thickness of the body and the opacity due to the presence of calcareous corpuscles in the reserve bladder. The tubule system is paired throughout; transverse commissures were never observed. The tubules on either side are collected into a pair of common trunks which seem to be very short and to empty directly into the reserve bladder at the level of the acetabulum (fig. 6 *j*). It is possible, however, that the association of these common trunks with the bladder (*sensu stricto*) in the hind-body may be more intimate as described by Faust (1922: Pl. 10, fig. 4) for *Tetracotyle orientales*. Following the terminology used by Looss (1894: 156) and Cort (1918: 132) the common trunks on either side give rise to a pair of anterior and a pair of posterior *primary collecting tubules* (*apt*, *ppct*). The posterior primary collecting tubules give rise by dichotomous branching to 32 pairs of accessory collecting tubules (*act*). The number of flame-cells with capillaries associated with each accessory collecting tubule was apparently two, but this point could not be definitely determined. The anterior primary collecting tubules are apparently associated with the same number of accessory collecting tubules, but the greater opacity of the anterior part of the body renders this point very uncertain. If these rather unsatisfactory observations are correct the total number of flame cells in *Tetracotyle communis* is 256. Faust (1922: 84) found that in *T. orientalis* each common trunk gives rise to 5

primary collecting tubules, each of which, based on a 5-fold dichotomy, drains a system of 64 flame-cells, making a total of 640 flame-cells for the entire body.

The reserve bladder of the present species was described by Hughes (1927: 255). It consists of an irregular continuous coarse-meshed network of spaces, not in the form of definite vessels. These spaces practically fill the entire fore-body, the only compact tissue found being that just below the cuticula and that contiguous to the lateral suckers, acetabulum, hold-fast organ, and various parts of the alimentary tract. In the lateral and dorsal parts of the body the parenchymal tissue is reduced to slender strands (figs. 7–10, *ps*). The spaces of the reserve bladder adjacent to the ventral body wall between the oral sucker and the acetabulum are smaller and the amount of parenchymal tissue among them greater than in other parts of the body. Even the more compact tissue about the acetabulum and lateral suckers contains small irregular ramifications of the reserve bladder.

An incomplete septum partially separates the spaces of the reserve bladder in the fore-body from the urinary bladder (*sensu stricto*) which occupies most of the space of the hind-body and which is divided anteriorly by a median septum. The posterior undivided portion of the urinary bladder communicates abruptly with the exterior through the excretory pore (fig. 5, 6, *ep*).

The spaces of the reserve bladder and urinary bladder are filled with a thin hyalin fluid, containing small granules of various sizes and of undetermined character, which moves freely in any direction in response to pressure or movements of the body. In addition to the small uniformly distributed freely-movable granules, the reserve bladder also contains larger spherical calcareous concretions, nearly uniform in size, lodged in the smaller spaces adjacent to the dorsal and ventral body-walls and distributed mainly anterior to the hold-fast organ. Although these concretions move within more or less restricted spaces, the pockets in which they occur are not comparable to the corpuscle-containing vesicles found in *Diplostomum volvens* von Nordmann since they communicate broadly with the larger spaces of the reserve bladder, evidenced by the fact that the concretions are easily separated from the parenchyma when the parasite is teased to pieces with a needle.

Tetracotyle diminuta sp. nov.*

The type specimens of this species were taken from 15 specimens of *Perca flavescens* Mitchill, varying in length from 7 to 12 inches, collected April 22, 1927, from Wampler Lake, Lenawee Co., Michigan, by Mr. T.

* Mounted type specimens of *T. diminuta* bear Catalog No. 260 in the Parasite Collection, Museum of Zoology, University of Michigan.

Huxley Langlois. The number of parasites varied from none to more than 100 per fish. In these fishes the parasites were found only on and about the heart, but they were not sought in other parts of the body. Similar parasites apparently conspecific with the type material were found in great numbers in *Perca flavescens* Mitchill and *Percopsis omisco-maycus* Walbaum from Douglas Lake, Michigan, during the summer of 1927. In these latter fishes the parasites were also sometimes found encysted in the walls of the eyes and in the adipose tissue of the eye-sockets. In *Perca flavescens* all of the tetracotyles were of this type but in *Percopsis omisco-maycus* a larger form, probably *Tetracotyle communis* was also found.

The ovate cyst (fig. 3) resembles that of *T. communis* in structure but it is exceedingly difficult to open without injury to the parasite. The cysts of 10 living specimens, not under pressure, varied in length from 0.33 mm. to 0.45 mm. (average 0.4 mm.) and in width from 0.28 mm. to 0.42 mm. (average 0.35 mm.). The cavities of the same cysts varied in length from 0.22 mm. to 0.32 mm. (average 0.29 mm.) and in width from 0.18 mm. to 0.27 mm. (average 0.23 mm.).

As in the preceeding species the parasite, within the cyst, is surrounded by a small amount of fluid containing excretory granules. Removed from the cyst it shows little activity, never using the oral sucker and acetabulum in locomotion. It remains viable for only a few hours after removal from the host and dies quickly under a cover-glass.

The general form of the body is essentially the same as that of *Tetracotyle communis*. The entire surface of the body is spinous. Specimens under pressure generally have the anterior part of the body, especially the lips of the oral sucker, covered with little mucous extrusions, uniform in shape and size and superficially resembling spines. These extrusions are associated with subcuticular, unicellular glands. The structures described by Claparède (1858, Pl. 8, fig. 6) as spines, were probably mucous extrusions. Six living specimens under cover-glass pressure varied in length from 0.286 mm. to 0.429 mm. (average 0.342 mm.) and in width from 0.204 mm. to 0.334 mm. (average 0.271 mm.). Ten mounted specimens varied in length from 0.155 mm. to 0.233 mm. (average 0.18 mm.) and in width from 0.134 mm. to 0.19 mm. (average 0.164 mm.).

The hold-fast organ (fig. 4, *hf*) is more rounded and relatively much larger than in *Tetracotyle communis*. In living specimens the cavity (fig. 4, *chf*) of the hold-fast organ is longitudinally elongate with bifurcate antero-lateral and postero-lateral ramifications, which are distinctly visible. In 6 living specimens under cover-glass pressure the hold-fast organ varied in length from 0.078 mm. to 0.15 mm. (average 0.122 mm.) and in width from 0.09 mm. to 0.18 mm. (average 0.135 mm.) and in 7 mounted specimens in length from 0.063 mm. to 0.105 mm. (average 0.075 mm.) and in width from 0.07 mm. to 0.105 mm. (average 0.085 mm.). The circular

acetabulum (fig. 4, *ac*) is relatively smaller than in *T. communis* and approximately the same size as the oral sucker. In 6 living specimens under cover-glass pressure it varied in diameter from 0.051 mm. to 0.063 mm. (average 0.056 mm.) and in 8 mounted specimens from 0.042 mm. to 0.053 mm. (average 0.048 mm.). The lateral suckers, similar in structure to those of *T. communis*, are frequently everted in living specimens under pressure and may be again retracted when the pressure is alleviated. In one living specimen under cover-glass pressure the cavity (fig. 4, *cls*) was 0.045 mm. long and its orifice (*mls*) 0.015 mm. in diameter. In 10 preserved specimens the length of the cavity of the lateral sucker varied from 0.033 mm. to 0.036 mm. (average 0.035 mm.). Unicellular glands, associated with the lateral suckers, like those in *T. communis* were not found.

The terminal, spheroidal, glandular oral sucker, in 6 living specimens under cover-glass pressure, varied in diameter from 0.051 mm. to 0.063 mm. (average 0.057 mm.) and in 8 mounted specimens from 0.042 mm. to 0.053 mm. (average 0.049 mm.). In 4 living specimens under cover-glass pressure, the pharynx, distinctly visible, varied in length from 0.02 mm. to 0.023 mm. (average 0.022 mm.) and in width from 0.015 mm. to 0.02 mm. (average 0.018 mm.). The esophagus and caecal furcae are similar to those of *T. communis*.

As far as observed the nervous, muscular, reproductive and excretory systems are the same as in *T. communis*. Of the nervous system only the supra-pharyngeal commissure and the proximal ends of the main trunks were seen. Of the primary excretory apparatus only a few scattered flame-cells and tubules were observed. The spaces of the reserve bladder contain irregularly shaped masses of a dense viscous liquid suspended in a thin watery fluid like that found in *T. communis*. Fine granules occur as in the preceeding species. Some of these move freely in the thin fluid and some are suspended in the viscous masses. The presences of the dense fluid in the spaces of the reserve bladder gives the parasite a somber aspect when viewed by transmitted light. The calcareous corpuscles are arranged as in *T. communis*.

Tetracotyle intermedia sp. nov.*

The type specimens of this species were taken from 2 specimens of *Prosopium quadrilaterale* (Richardson), about 12 inches long, collected in the latter part of April, 1927, from Lake Huron, near St. Ignace, Michigan. Several *Leucichthys artedi* Le Sueur from the same locality collected in May, 1927 contained parasites of apparently the same species. In all of these, the fish parasites were found in the pericardium.

The cyst (fig. 2), similar to that of *T. diminuta*, in 10 living specimens

* Mounted type specimens of *T. intermedia* bear Catalog No. 259 in the Parasite Collection, Museum of Zoology, University of Michigan.

not under pressure, varied in length from 0.55 mm. to 0.75 mm. (average 0.61 mm.) and in width from 0.4 mm. to 0.56 mm. (average 0.48 mm.). The cavities of the same cysts varied in length from 0.34 mm. to 0.45 mm. (average 0 41 mm.) and in width from 0.25 mm. to 0.32 mm. (average 0.26 mm.). The parasite, freed from the cyst, resembles *T. diminuta* in respect to movements, viability, and general body form. Mucous extrusions about the oral sucker were seen in specimens under pressure, but they were less abundant than in *T. diminuta*. Five living specimens, under cover-glass pressure, varied in length from 0.37 mm. to 0.59 mm. (average 0.48 mm.) and in width from 0.3 mm. to 0.6 mm. (average 0.44 mm.). Eight mounted specimens varied in length from 0.3 mm. to 0.42 mm. (average 0.38 mm.) and in width from 0.25 mm. to 0.33 mm. (average 0.3 mm.).

The hold-fast organ, similar in shape to, but relatively smaller than, that of *T. diminuta* has a rather simple transversely elongate cavity like that of *T. communis*, the innermost recesses of which are scarcely visible in living specimens. In 5 living specimens under cover-glass pressure the hold-fast organ varied in length from 0.11 mm. to 0.16 mm. (average 0.13 mm.) and in width from 0.15 mm. to 0.2 mm. (average 0.18 mm.) and in 6 mounted specimens, in length from 0.07 mm. to 0.08 mm. (average 0.074 mm.) and in width from 0.09 mm. to 0.11 mm. (average 0.105 mm.).

The acetabulum is slightly elongate transversely and a little larger than the oral sucker. In 5 living specimens under cover-glass pressure it varied in length from 0.045 mm. to 0.062 mm. (average 0.055 mm.) and in width from 0.06 mm. to 0.081 mm. (average 0.07 mm.) and in 8 mounted specimens, in length from 0.052 mm. to 0.066 mm. (average 0.057 mm.) and in width from 0.056 mm. to 0.077 mm. (average 0.066 mm.). The lateral suckers are similar in structure and behavior to those of *T. diminuta*. In 10 preserved specimens the length of the cavity of the lateral sucker varied from 0.035 mm. to 0.052 mm. (average 0.049 mm.) . The ventroterminal oral sucker, in 4 living specimens under cover-glass pressure, varied in length from 0.06 mm. to 0.075 mm. (average 0.066 mm.) and in width from 0.054 mm. to 0.06 mm. (average 0.058 mm.) and in 8 mounted specimens, in length from 0.043 mm. to 0.063 mm. (average 0.059 mm.). In three living specimens under cover-glass pressure the small plainly visible pharynx was 0.03 mm., 0.024 mm., and 0.03 mm. long and 0.024 mm., 0.018 mm., and 0.015 mm. wide respectively. Concerning the rest of the digestive system, the nervous, muscular, reproductive and excretory systems the statements made in the description of *Tetracotyle diminuta* are also true of *Tetracotyle intermedia*.

Discussion

It is possible that the three groups of specimens herein described as

distinct species are merely age variations of the same form. That these forms are distinct species, however, seems probable for the following reasons: (1) they were collected too early in the spring, probably, to represent recent infections since it is doubtful that strigeid cercariae are produced when their snail hosts are in very cold water; (2) they were collected at about the same time of year and might be expected to be in comparable stages of development; (3) they fall into 3 distinct non-intergrading size groups; (4) they have constant morphological differences which have already been discussed. Furthermore, that *Tetracotyle diminuta* and *T. communis* are distinct species is evidenced by the fact that of the fishes examined at Douglas Lake, collected at the same time and from the same locality, *Perca flavescens* was parasitized with the one, *Catostomus commersonnii* with the other, and *Percopsis omisco-maycus* with both forms, conditions which could hardly obtain if the two are conspecific. But this evidence is not conclusive; these fishes were examined only during the summer; assuming that these two types of parasites *are* conspecific the differences in form which they show in the three species of fish might be explained in terms of the habits of the fishes themselves or of inter-host-parasite relationships. For example the cercaria of this form might be able to penetrate *Perca flavescens*, metamorphose and become encysted but not to maintain itself long enough to attain its maximum development. If this be true, however, one should have been able to find among the viable cysts some which were dead and disintegrating, but such were not found. On the other hand *Catostomus commersonnii*, in early life may be susceptible to the penetration of this parasite and thereafter become immune because of an intrinsic physiological adaptation or a change in habits which removes it from the range of infective cercariae, an explanation which at once accounts for the occurrence of the parasite in relatively small numbers and in uniformly large size in this host. Whether these three forms of Tetracotyle are identical, can be determined, probably, only by the use of experimental data.

Diplostomum parvulum Stafford, described and reported from cysts about the pharynx of *Semotilus bullaris* by Stafford (1904: 494) and referred to by Faust (1917: 66) as *Diplostomulum parvulum*, and by Mataré (1910: 497) as *Tetracotyle parvula*, may be identical with *Tetracotyle communis*. The few measurements given and general features described compare favorably with those of my smaller mounted specimens of *T. communis*. The brief original description makes no mention of lateral suckers, but in a cursory study, such structures might easily have been overlooked.

In general, the so-called "suctorial-pocket" (Faust, 1922: Pl. 9, fig. 1, *P*) which, in Tetracotyles from other kinds of hosts, is usually a deeply

cup-shaped concavity on the ventral side of the fore-body, is very shallow or wanting in those tetracotyles which have been described from fishes.

The study of our specimens of *Tetracotyle variegata*, *Tetracotyle sogdiana* and *Tetracotyle percae-fluviatilis* and the description of the excretory system of *Tetracotyle echinata* presented by Claparède (1858: 102) show that in these species the reserve bladder is essentially like that of *Tetracotyle communis*.

Comparative Synopsis of other Tetracotyles from Fish Hosts

1. *Tetracotyle variegata* (Creplin) Mihi

Synonym: *Tetracotyle ovata* von Linstow (1877: 192)

Descriptions of this species based on the original description by von Linstow (1877: 192; Pl. xiv, fig. 24) have been published by Lühe (1909: 170) and Faust (1918: 77). In these descriptions and the reports of Braun (1892: 797 and 1894b: 167), Otterstrøm (1915: 221) and Zandt (1924: 242) the species has been listed from *Acerina cernua*, *Abramis brama*, *Blicca bjoerkna*, *Osmerus eperlanus*, *Perca fluviatilis*, *Aspius alburnus*, *Esox lucius*, and *Phoxinus laevis*, encysted in the peritoneum of the body cavity, digestive tract, ovaries, air-bladder, pericardial cavity and in the eye muscles, from various parts of Europe. A feeding experiment performed by the Ehrhardt brothers (Braun 1894a: 681; 1894b: 166; 1894c: 755) demonstrated that *Tetracotyle ovata* from *Acerina cernua* develops into *Holostomum variegatum* (Creplin) in *Larus ridibundus* and *Sterna hirundo*. Thus the specific name *ovata* becomes a synonym of *variegatum*.

Tetracotyle variegata resembles the species herein hescribed, particularly *Tetracotyle communis* more closely than do any of the other species of Europe and Asia. According to the reports mentioned above and my studies of the specimens of *Tetracotyle variegata* sent to us by Professor Scheuring I find that *Tetracotyle communis* differs from *T. variegata* as tabulated below.

Tetracotyle communis	*Tetracotyle variegata* (Creplin)
1. Occurs chiefly about heart of host; rarely in other parts of body.	Found commonly in peritoneal coverings of body cavity, digestive tract, air-bladder and ovaries, as well as in pericardial cavity of host.
2. Cyst wall thick and lamellar (fig. 1)	Cyst wall thin (von Linstow, 1877: 192)
3. Acetabulum, in specimens mounted *in toto*, a little larger than the hold-fast organ.	Acetabulum, in specimens mounted *in toto*, a little smaller than hold-fast organ.
4. Parenchymal tissue contiguous with cuticle and in strands and mesenteries associated with hold-fast organ and acetabulum relatively thin.	Parenchymal tissue contiguous with cuticle and in strands and mesenteries associated with the hold-fast organ and acetabulum, relatively thick.
5. Large subcuticular unicellular glands, which are not associated with lateral suckers, not present.	Large subcuticular unicellular glands, which are not associated with lateral suckers, present and especially abundant on the ventral side.

2. *Tetracotyle echinata* Diesing (1858: 367)

Synonymy: Distome from *Cyprinus idus* of Dujardin (1845: 463)
Tetracotyle from *Cyprinus idus* of Moulinié (1856: 233)
Tetracotyle from *Acerina cernua* of Claparède (1858: 103)
Tetracotyle typica Diesing (1858: 366) ex parte
Tetracotyle from *Leuciscus dobula* and *Cyprinus carpio* of Pagenstecher (1857: 246)

Diesing applied the name *Tetracotyle echinata* to the form described and figured by Claparède (1858: Pl. VIII, fig. 6) and regarded the one described by Dujardin as probably identical with it. Moulinié regarded his material from *Cyprinus idus* to be identical with that of Dujardin. The tetracotyles found by Pagenstecher in *Leuciscus dobula* and *Cyprinus carpio* are listed by Lühe as belonging probably to this species. Other descriptions of *T. echinata* have been compiled by Lühe (1909: 169) and Faust (1918: 76). It is described as having scattered dermal spines. These are probably mucous extrusions such as I have found in *Tetracotyle diminuta* and *T. intermedia*. *Tetracotyle echinata* is characterized by the large size of the hind-body, plainly visible in dorsal or ventral view, causing the outline of the body to be narrowed posteriorly. The excretory system (reserve bladder) of *Tetracotyle echinata* was described in some detail by Claparède (1858: 102). Except that he found the reserve bladder to consist of two parts (right and left halves) his statements agree with my own findings in the species described in this paper and in *T. variegata*.

3. *Tetracotyle percae-fluviatilis* von Linstow.

Synonymy:

Tetracotyle from *Perca fluviatilis* Moulinié (1856: 230)
Tetracotyle typica Diesing Diesing (1858: 366)
"Tetracotyle Percae fluviatilis *Moulinié*" Diesing (1858: 366) (listed as belonging to *Tetracotyle typica* Diesing).
Tetracotyle Percae fluviatilis Moulinié von Linstow (1877: 192)
Tetracotyle Percae Zschokke (1884: 163)
Tetracotyle Percae Monticelli (1888: 7)
Tetracotylus percae fluviatilis (Moulinié) Piana (1898a: 396)
Tetracotyle Percae fluviatilis Moulinié) Piana (1898b: 378)
Tetracotyle percae fluviatilis v. Linst. Lühe (1909: 170)
Tetracotyle percae fluviatilis Moul. Stiles and Hassal (1908: 370)
Tetracotyle musculorum percae Mataré (1910: 496)
Tetracotyle percae-fluviatilis Moulinié Faust (1918b: 76)
Tetracotyle perca fluviatilis v. Linstow Zandt (1924: 242)
Tetracotyle percae fluviatilis Moulinié Mathias (1925: 17)

This form was originally described but not named by Moulinié. The expression "Tetracotyle Percae fluviatilis *Moulinié*" of Diesing was

probably not intended for a name, but was so used by von Linstow. Descriptions based on those of Moulinié and von Linstow have been published by Piana, Lühe and Faust as cited above and by Zschokke (1884: 205). A figure of this species was given by von Linstow (1877: Pl. XIV, fig. 23). The species occurs chiefly in peritoneal cysts and has been reported only from *Perca fluviatilis* in Europe. That it is specific for *Perca fluviatilis* is suggested by this statement of Piana (1898b: 378). "Senza timore esagerare, posso affemare che più del 90 per cento dei pesci persici da me esaminati erano infestati da tetracotili. Negli altri pesci del lago di Varese non mi fu dato di trovare una sol volta un simile parassita." *T. percae-fluviatilis* is uniquė among the fish-inhabiting species in that the lateral suckers are much larger than the oral suckers.

In regard to a feeding experiment performed to determine the definitive form of *Tetracotyle percae-fluviatilis* I quote from Piana (1898b: 383). "Solo notero che fra i tetracotili trovati viventi e spogli di capsula nel proventricolo di un'anitra, a cui da un'ora aveva somministrato alcune vesciche natatorie di pesci persici, ne notai uno il quale, nel posto dell'orifizio di uno degli infundibuli laterali, presentava sporgente un zaffo in forma di spattula e, in corrispondenza alla parta anteriore della superficie ventrale corpo, una forte depressione in modo da convincermi maggiormente, che il trematode a completo sviluppo dirivante dal *Tetracotyle Percae fluviatilis* sia una specie di Conchosomum." This is a very interesting speculation, since, if correct, it shows that the genus Alaria Schrank (synonym, Conchosomum Raillet) develops from a Tetracotyle. The evidence presented, however, is insufficient to establish the conclusion.

4. *Tetracotyle sogdiana* Pavlovsky and Anitchkov (1922).

This species, found encysted in the pancreas of *Schizothorax intermedius* collected from the River Zeravshan in Turkestan by Professor E. N. Pavlovsky in 1908, was described by Pavlovsky and Anitchkov (1922). It differs from the other fish-inhabiting Tetracotyles by having the acetabulum located anterior to the middle of the body and by the presence of longitudinal invaginations in the dorsal body wall. The ventral invaginaation posterior to the acetabulum is doubtless the hold-fast organ and the statement that the rudiments of reproductive organs are associated with it (Pavlovsky and Anitchkov, 1922: 310) is probably in error. Analogous errors made by Faust in his descriptions of strigeid metacercariae have been adequately corrected by Szidat (1924: 310), Ashworth and Bannerman (1927: 162) and Hughes (1928: 46).

Acknowledgements

I wish here to express my grateful appreciation to Professor George R. La Rue of the Zoölogy Department and Director of the Biological

Station, University of Michigan, by whom this study was suggested and under whom it has been conducted.

I am indebted to Professor L. Scheuring of the Bayr. Biol. Versuchsanstalt für Fischerei, München, Professor E. N. Pavlovsky of the Military Academy of Medicine, Leningrad, and Dr. Ferdinand Zandt of the Anstalt für Bodenseeforschung der Stadt Konstanz for aid in obtaining specimens of *Tetracotyle variegata*, *Tetracotyle sogdiana* and *Tetracotyle percae-fluviatilis* respectively.

My thanks are due to Dr. Carl L. Hubbs of the Museum of Zoölgy, University of Michigan, for aid in the identification of fishes; to Mr. T. Huxley Langlois, Michigan State Fish Pathologist, for aid in securing the type specimens of *Tetracotyle diminuta* and to Mr. Tuenis Vergeer of the Zoölogy Department, University of Michigan, for aid in securing the type specimens of *Tetracotyle communis* and *Tetracotyle intermedia*.

Literature Cited

Ashworth, A. W. and Bannerman, J. C. W. 1927.—On a tetracotyle (T. phoxini) in the brain of the minnow. Trans. R. Soc. Edinburgh, 55: 159–172, 8 text figs., 1 pl.

Braun, M. 1892.—Vermes. In Bronn's Kl. u. Ord. d. Thier-Reichs., 4, Abt. 1A. 925 pp. Leipzig.

1894a.—Helminthologische Notizen. IV. Zur Ent wicklungsgeschichte der Holostomiden. Centralbl. f. Backt. u. Parasitenk., 15: 680–682.

1894b.—Zur Entwicklungsgeschichte der Holostomiden. Nach den Untersuchungen der Herren Alfred und Oscar Ehrhardt mitgetheilt. Zool. Anz., 17: 165–167.

1894c.—Idem. Zool. Centralbl., 1 (19–20): 755.

Brown, A. W. 1899.—On *Tetracotyle petromyzontis*, a parasite of the brain of Ammocoetes. Quart. Jour. Micr. Science, n. s. 41: 489–498, 1 pl.

Claparède, E. 1858.—Ueber die Kalkkörperchen der Trematoden und die Gattung Tetracotyle. Zeitschr. f. wissensch. Zool. 9: 99–105, 1 pl.

Cort, W. W. 1918.—The excretory system of *Agamodistomum marcianae* (La Rue), the agamodistome stage of a forked-tailed cercaria. Jour. Parasitol., 4: 130–134, 3 figs.

Diesing, K. M. 1858.—Revision der Myzhelminthen. Abt: Trematoden. Sitzungsb. d. k. Akad. d. Wissensch. Math.-Naturw. Kl., 32 (23): 307–390, 2 pls.

Dujardin, F. 1845.—Histoire naturelle des Helminthes ou vers intestinaux. 654 pp. Paris.

Faust, E. C. 1917.—Life History Studies on Montana trematodes. Ill. Biol. Monogr., 4 (1): 7–120, 9 pls.

1918.—The anatomy of *Tetracotyle iturbei* Faust, with a synopsis of described tetracotyliform larvae. Jour. Parasitol., 5: 69–79, 1 pl.

1922.—Phases in the life history of a holostome *Cyathocotyle orientalis* nov. spec. with notes on the excretory system of the larva. Jour. Parasitol., 8: 78–85, 2 pls.

Fraipont, J. 1880.—Recherches sur l'appareil excréteur des trématodes et des cestodes. Archives de Biol., 1: 415–456, 2 pls.

Hughes, R. C. 1927.—Studies on the trematode family Strigeidae (Holostomidae) No. VI. A new metacercaria *Neascus ambloplitis*, sp. nov. representing a new larval group. Trans. Am. Micr. Soc., 46: 248–267, 2 pls.

1928.—Idem No. VII. *Tetracotyle pipientis* Faust. Idem, 47: 42–53, 1 fig.

von Linstow, O. 1877.—Enthelminthologica. Archiv. f. Naturg., 43: 173–198, 3 pls.

Looss, A. 1894.—Die Distomen unserer Fische und Frösche. Bibl. Zool., 16: 1–296, 9 pls.

Lühe, M. 1909.—Parasitische Plattwürmer I. Trematoden. Brauer's Süsswasserfauna Deutschlands. Heft 17. 217 pp.

Mataré, F. 1910.—Ueber eine neue Tetracotyle im Hirn von *Phoxinus laevis*. Zeitschr. f. wissensch. Zool., 94: 488–450. 3 figs., 1 pl.

Mathias, P. 1925.—Recherches expérimental sur le cycle évolutif de quelques trématodes. Bull. Biol. Fr. Bel., 59: 1–124, 4 pls., 13 figs.

Monticelli, F. S. 1888.—Saggio di una morologia dei trematodi. Tesi per ottenere la privata dozenza in zoologia nella R. Università di Napoli. 130 pp. Napoli.

Moulinié, J. J. 1856.—De la reproduction chez les trématodes endoparasites. Mém. de l'Inst. Nation. genevois., 3: 7–278, 6 pls.

Otterstrøm, C. V. 1915.—Ichthyologiske Nottiser. I. Hocke doden i Easom. Sø 1913. Vidensk. Meddel. Densk. nat. Foren., 66: 221–225, fig.

Pagenstecher, H. A.—1857.—Ueber Erziehung des *Distoma echinatum* durch Fütterung. Arch. f. Naturg., 23: 244–251.

Pavlovsky, E. N. and Anitchkov, N. N. 1922.—*Tetracotyle sogdiana*, a new trematode parasite of the fish *Schizothorax intermedius* with a description of adhesive peritonitis produced by the parasite in its host. Parasitology, 14: 309–314, 1 pl.

Piana, G. P. 1898a.—Beobachtungen über das Vorkommen des *Tetracotylus percae fluviatilis* (Moulinié) und einige Krankheitserscheinungen des Barsches. [Abstract]. Allg. Fischerei-Zeitung, 23 (N.F. 13): 396–397.

1898b.—Osservazioni sul *Tetracotyle percae fluviatilis* Moulinié e su alcuni fenomeni verificati nei pesci persici. Atti Soc. Ital. di Sc. Nat. (etc.), 37: 378–387.

Stafford, J. 1904.—Trematodes from Canadian fishes. Zool. Anz., 27: 481–495.

Stiles, C. W. and Hassal, A. 1908.—Index Catalogue of Medical and Veterinary Zoology. Trematoda and Trematode Diseases. Bull. 37, U. S. Hygienic Laboratory. 401 pp. Washington.

Szidat, L. 1924.—Beiträge zur Entwicklungsgeschichte der Holostomiden, I. Zool. Anz., 58: 299–314, 9 figs.

Zandt, F. 1924.—Fischparasiten des Bodensees. Centralbl. f. Backt. u. Parasitenk. (Orig.) 92: 225–271, 33 figs.

Zschokke, F. 1884.—Recherches sur l'organisation et la distribution zoologique des vers parasites des poissons d'eau douce. Arch. de Biol., 5 (2): 153–241, 2 pls.

Explanation of Figures

ac—acetabulum
an—anterior nerve
at—accessory collecting tubule
apt—anterior primary collecting tubule
ban—branches of anterior nerve
c—cuticle
cg—unicellular glands associated with lateral sucker.
chf—cavity of hold-fast organ
cls—cavity of lateral sucker
cmf—circular muscle fibers
dvmf—dorso-ventral muscle fiber
ep—excretory pore
hb—hind-body
hf—hold-fast organ
hfm—hold-fast mesenteries
int—digestive caecum
j—apparent juncture of primary excretory apparatus and reserve bladder
lmf—longitudinal muscle fibers
ls—lateral sucker
m—mouth
mls—external meatus of lateral sucker
oe—oesophagus
ohf—external meatus of hold-fast organ
os—oral sucker
ph—pharynx
pn—posterior nerve
ppct—posterior primary collecting tubule
spnc—supra-pharyngeal nerve commissure
srb—spaces of reserve bladder
tpea—tubules of primary excretory apparatus

Plate LV

Figures 1 to 6 were drawn from living specimens. The guide line applies only to fig. 7

Fig. 1. Cyst of *Tetracotyle communis* from the pericardial cavity of *Stizostedion canadense griseum* De Kay, 44 X.

Fig. 2. Cyst of *Tetracotyle intermedia* from pericardial cavity of *Prosopium quadrilaterale* (Richardson). 44 X.

Fig. 3. Cyst of *Tetracotyle diminuta* from pericardial cavity of *Perca flavescens* Mitchill. 44 X.

Fig. 4. Ventral view of *Tetracotyle diminuta*. Approximately 63 X.

Fig. 5. Side view of *Tetracotyle communis*. Approximately 63 X.

Fig. 6. Ventral view of *Tetracotyle communis*. Approximately 90 X.

Fig. 7. Cross-section through the pharynx of a Tetracotyle, similar to *T. communis*, from *Catostomus commersonnii*. Drawn with the aid of a camera lucida.

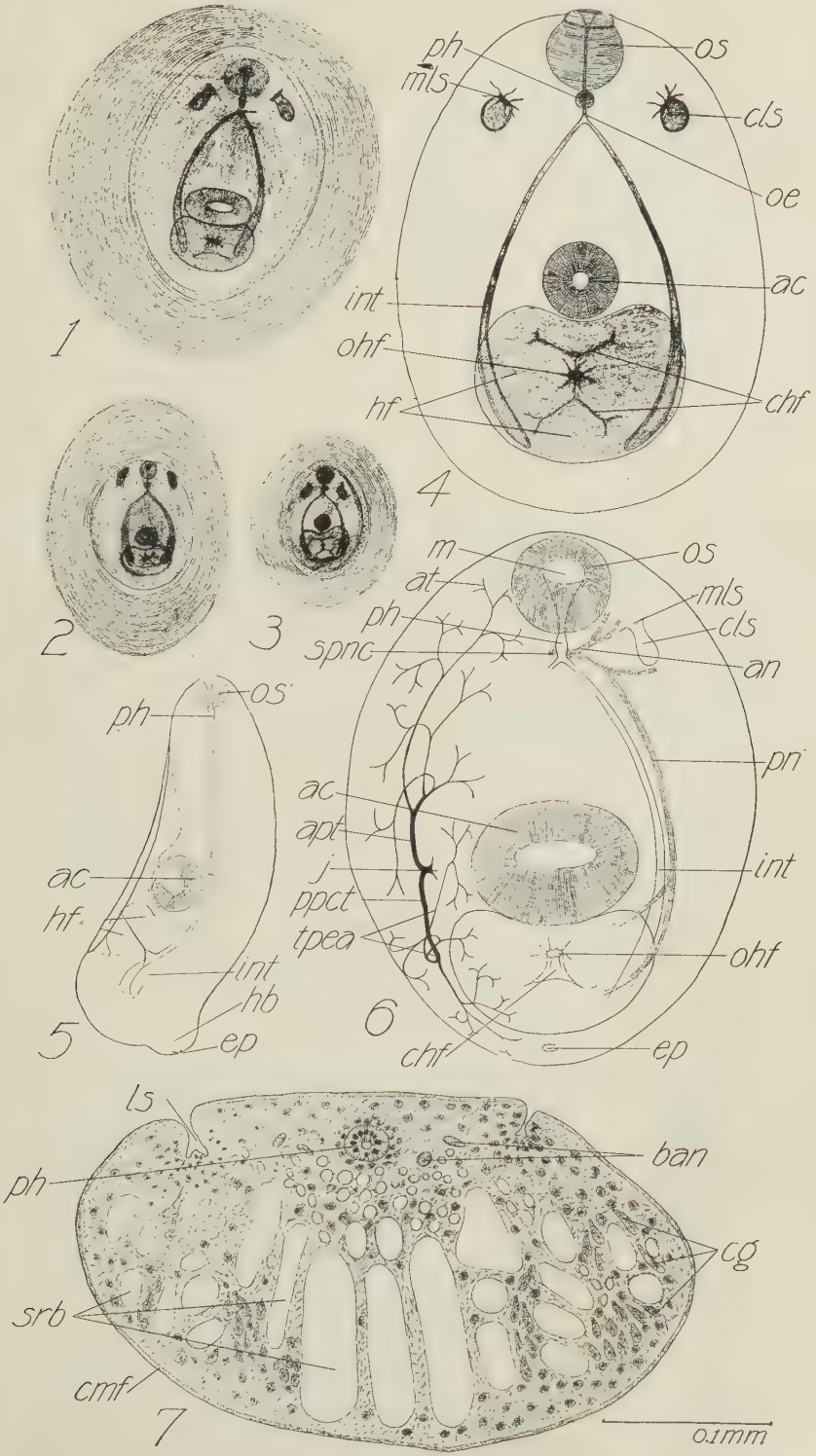
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PLATE LV

Plate LVI

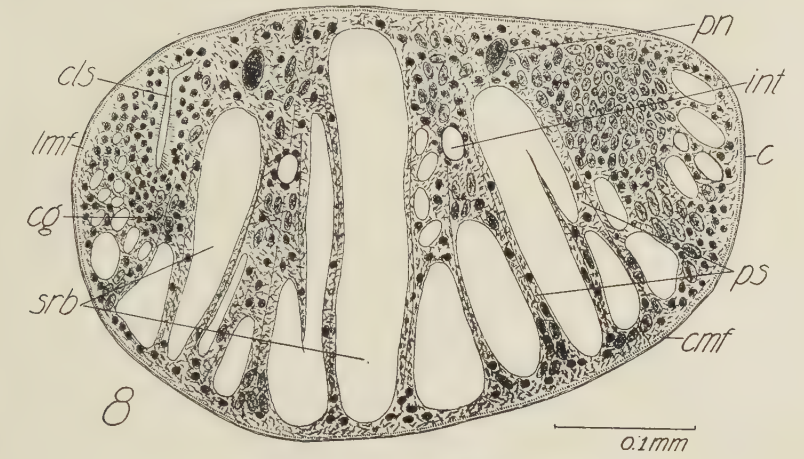
Figures 8, 9, and 10 are cross-sections of *Tetracotyle communis* from *Stizostedion canadense griseum*. Drawn with the aid of a camera lucida.

Fig. 8. Section, slightly oblique, through the cavity of one of the lateral suckers and the region immediately behind the other.

Fig. 9. Through the acetabulum.

Fig. 10. Through the hold-fast organ.

Fig. 11. The lateral sucking cup of *Tetracotyle communis* as it appears in preserved specimens. Free-hand drawing of ventral aspect.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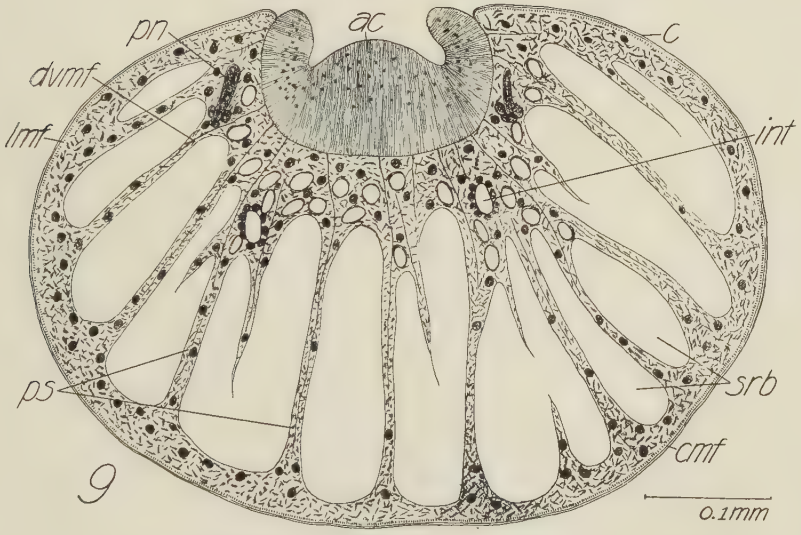
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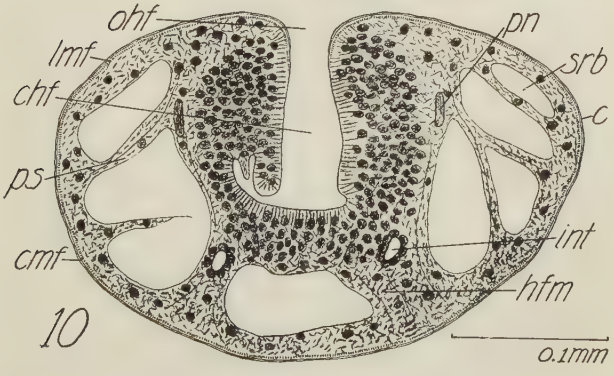
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PLATE LVI

Number 202 June 18, 1929

OCCASIONAL PAPERS OF THE MUSEUM OF ZOOLOGY

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. XIV. TWO NEW SPECIES OF DIPLOSTOMULA[1]

By R. Chester Hughes

The purpose of this article is to present descriptions of two new species of strigeid metacercariae, together with a discussion of the characteristics and the nomenclature of the larval group (Diplostomulum) to which they belong and a comparative synopsis of the other known species.

Diplostomulum browni, sp. nov.[2]

During the summer of 1927 a single specimen of *Lymnaea emarginata angulata* Sowerby, heavily infected with a new

[1] Contribution from the Biological Station and from the Department of Zoology, University of Michigan. This is the fourteenth of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

[2] Specimens referred to in this paper have the following numbers in the parasite collection of the Museum of Zoology of the University of Michigan: *Diplostomulum browni:* type slide, specimens mounted *in toto*, No. 261; paratypes mounted in sections, No. 262; paratypes in alcohol, No.

species of Diplostomulum larva, *Diplostomulum browni,* was found and presented to me by Dr. Harold W. Brown. This snail, taken from the northwestern part of Douglas Lake, Cheboygan County, Michigan, contained several hundred specimens of the present parasite together with a number of sporocysts producing an undetermined species of strigeid cercaria. The diplostomula were not encysted and occurred scattered throughout the soft parts of the host; indeed, many were found within the sporocysts mentioned above. These were also not encysted. During the summer a great many snails of several species were carefully examined for parasites by a number of investigators at the Biological Station, but no others were found to be infected with diplostomula. So far as I am aware this is the first record of the occurrence of a diplostomulum parasitic in snails.

Careful studies were made of both living and preserved material. The study of living specimens was especially useful in working out the details of the excretory system, the vessels of which are so small and delicate that it is almost impossible to trace them in preserved animals. The minutiae of the excretory pattern are most clearly visible in living worms mounted in water and under considerable pressure of a coverglass. It is regrettable that an opportunity for making a large series of measurements of living specimens was not afforded. For whole mounts and sections, specimens were fixed in sublimate-acetic and stained *in toto* in Ehrlich's acid haematoxylin made very dilute with 70 per cent alcohol. The sections were decolorized on the slide and counterstained with eosin.

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210. *D. corti:* type specimen mounted *in toto,* No. 288; paratypes mounted *in toto,* Nos. 289–294; paratypes mounted in sections, Nos. 295–300. *D. spathaceum:* specimens mounted *in toto,* No. 276; specimens mounted in sections, No. 277. *D. petromyzi-fluviatilis:* specimens mounted *in toto,* No. 269; specimens mounted in sections, No. 270. *D. phoxini:* specimens mounted *in toto,* No. 275. *Tetracotyle sp.* identified by Dr. F. Zandt as *Diplostomum cobitidis:* specimens mounted *in toto,* No. 250.

For purposes of comparison specimens of *Diplostomulum spathaceum* (Rudolphi) (synonym, *Diplostomum volvens* von Nordmann), *Diplostomulum phoxini* (Faust), presented to Professor La Rue by Professor L. Scheuring of München, and *Diplostomulum petromyzi-fluviatilis* (Diesing), presented by Professor E. S. Goodrich of Oxford, were stained and mounted as outlined above.

The body of the parasite comprises two well-defined regions, the fore- and hind-bodies. In living specimens the fore-body is thin, foliaceous, ovate in outline, longitudinally elongate, slightly concave ventrally and slightly convex dorsally. In preserved specimens the concavity of the ventral surface is lost. The relatively small hind-body is present as a hemispherical appendage on the dorsal side of the posterior end of the fore-body. The oral sucker (Fig. 5*os*) is antero-terminal, with the mouth opening directed ventrad. The so-called lateral suckers (Fig. 5*ls*) are usually extended as short papillae, but may be withdrawn to form shallow cup-shaped depressions. In preserved specimens the lateral suckers usually appear as slight inconspicuous projections of the antero-lateral margins of the body. The ventral sucker (Fig. 5*ac*), located at the anterior end of the posterior half of the fore-body, is slightly smaller than the oral sucker. The hold-fast organ (Fig. 5*hf*), situated immediately behind the acetabulum, is somewhat larger than the acetabulum. The cuticula of the entire body is uniformly armed with minute quincuncially arranged spines. Four living worms, under cover-glass pressure, varied in length from 0.247 mm. to 0.277 mm. (average 0.262 mm.), and in width from 0.182 mm. to 0.211 mm. (average 0.189 mm.). The principal measurements of ten specimens mounted *in toto* are given in Table I.

The parasites were very active when removed from the tissues of the snail and placed in water. The movements consisted of alternately lengthening and shortening the body by means of which processes no spatial locomotion was accomplished. Occasionally, however, a specimen was observed crawling on the bottom of the container, after the manner of

TABLE I

Measurements in Millimeters of Ten Mounted Specimens of Diplostomulum browni

	Minimum	Maximum	Average
Body			
length	0.162	0.233	0.206
width	0.106	0.141	0.127
Oral sucker			
diameter	0.028	0.035	0.033
Pharynx			
length	0.021	0.028	0.022
length	0.024	0.032	0.027
Acetabulum			
width	0.028	0.035	0.032
length	0.042	0.053	0.046
Hold-fast organ			
width	0.035	0.053	0.047

a leech, or on the under side of the surface film of the water, using the oral sucker and acetabulum for attachment.

The digestive tract comprises the usual parts. The pharynx (Fig. 5*ph*) is large and muscular, the prepharynx and oesophagus (*oe*) short. The caecal furcae (Figs. 5, 8*int*) extend well into the hind-body. In living specimens the caeca often contain irregularly shaped granules of solid substance which surge back and forth in response to movements of the body. Of the nervous system only the suprapharyngeal commissure (Fig. 5*sp*) and the anterior (*an*) and posterior (Figs. 5, 8*pn*) pairs of nerve trunks were observed. The posterior trunks were traced back to the regions postero-lateral to the hold-fast organ. The reproductive system is represented by a single heavily staining mass of cells (Fig. 5*rf*) situated in the proximal part of the hind-body in the septum between the halves of the urinary bladder. The body is provided with subcuticular transverse (Fig. 8*cmf*) and longitudinal (*lmf*) muscle fibers. Other muscle fibers were not observed. Scat-

tered throughout the parenchyma are large unicellular glands the ducts of which communicate directly with the surface. In living specimens the mouths of the ducts of these glands are plugged by papilliform accretions of the exudate. A distinct compact adhesive gland associated with the hold-fast organ was not observed.

The excretory system closely resembles that of *Diplostomulum spathaceum* (Rudolphi) which was studied by Claparède (1858) and more carefully by Fraipont (1880) and Zandt (1924). The primary excretory apparatus, consisting of flame cells and associated tubules, is greatly obscured by the thickness of the animal and the extensive development of the reserve bladder. Many flame cells and scattered tubules were observed, but not in sufficient detail to give any definite idea of their arrangement. The bilobate urinary bladder (Fig. 7*ub*) occupies the greater part of the hind-body. Its two halves, separated anteriorly by a median septum, are joined posteriorly and communicate with the dorso-terminal excretory pore (*ep*) through a short duct (*ed*). The halves of the urinary bladder, inflated in the hind-body, extend into the fore-body as narrow tubules, to the region antero-lateral to the acetabulum. At the level of the hold-fast organ (*j*) the tubular portions of the urinary bladder receive the common trunks of the *primary excretory apparatus.* The common trunks are short and each bifurcates, giving rise to an *anterior* and a *posterior primary collecting tubule* (*apt, ppt*). These were traced to the anterior and posterior reaches of the body, respectively, but their exact relationship to the flame cells was not determined. The principal tubules of the primary excretory apparatus lie dorsal to those of the reserve bladder. The fact that the flame cells in *Diplostomulum spathaceum* are associated with a pair of longitudinal tubules, not related directly to the vesicles containing calcareous corpuscles, was observed by Fraipont (1880: 432) and Zandt (1924: 237). The relationship, however, of these tubules to the urinary bladder was not clearly understood. Fraipont considered that each longitudinal tubule associated with flame

cells communicates with the rest of the excretory system at two and probably three different points, whereas Zandt considered that they empty directly into the posterior portions of the urinary bladder.

In the regions antero-lateral to the acetabulum the tubular portions of the urinary bladder give rise to two pairs of vessels which connect with the tubules of the reserve bladder. These are the *lateral collecting vessels* (Fig. 7*lc*) and the *posterior transverse commissural vessels* (*pc*). The *posterior transverse commissural vessels* extend mesad to join the *median dorsal vessel* (*md*). The lateral collecting vessels extend antero-laterad and soon bifurcate, giving origin to the *antero-lateral* (*al*) and *postero-lateral vessels* (*pl*). The antero-lateral vessels extend forward to the regions near the oral sucker where they are joined to the anterior end of the median dorsal vessel by the *anterior transverse commissural vessels* (*ac*). The postero-lateral vessels extend posteriad, lateral to the tubular portions of the urinary bladder, and end blindly near the median line in the posterior part of the fore-body. The median dorsal vessel (*md*), formed by the union of the anterior transverse commissural vessels, extends posteriorly, ending blindly dorsal to the hold-fast organ. The median dorsal, anterior transverse commissural, antero-lateral and postero-lateral vessels receive many short branches, often dendritic, the distal termini of which are inflated as small spherical vesicles each containing a spherical calcareous concretion (*cc*). The vesicles and the calcareous corpuscles contained in them are somewhat uniform in size and are arranged in two layers, near the ventral and dorsal body-walls. The number of such vesicles and the size of the corpuscles vary greatly in different individuals.

Diplostomulum corti, sp. nov.

This description is based upon notes and sketches and a number of preserved specimens, some sectioned and some mounted *in toto,* kindly given to me by Professor W. W. Cort, who made a preliminary study of this form but neglected to

publish his discoveries. The parasites upon which the observations were made were found encysted in the muscles and particularly at the bases of the fins of *Ameiurus melas* (Rafinesque) and *Ameiurus nebulosus* (Le Sueur) obtained from the Illinois River during November and December, 1909. Five of fifteen fish examined were found to be infected, one so heavily that the muscles were almost entirely filled with the trematodes. The cyst was composed of a loose sac of connective tissue, probably entirely of host origin. When removed from the cysts the larvae moved actively, but the nature of the movement was not recorded.

In form *Diplostomulum corti* (Fig. 6) resembles *Diplostomulum browni,* but is considerably larger. The ventral surface of the fore-body is more concave, the posterior end is curved ventrad forming a transverse fold. The hind-body is much larger. The acetabulum (*ac*) and greater part of the hold-fast organ (*hf*) lie anterior to the middle of the body and the lateral suckers (*ls*) are much more deeply cupped.

TABLE II

Measurements in Millimeters of Preserved Specimens of Diplostomulum corti

Number of Specimen	1	2	3	4	Average
Body					
length	0.81	0.70	0.85	0.88	0.81
width	0.50	0.40	0.47	0.53	0.475
Distance of acetabulum from anterior end	0.28	0.25	0.29	0.26	0.27
Oral sucker, diameter	0.066	0.062	0.066	0.072	0.067
Pharynx					
length	0.053	.....	0.045	0.040	0.045
width	0.036	.....	0.026	0.038	0.033
Acetabulum, diameter	0.073	0.073	0.073	0.083	0.076
Intestinal caecum, length	0.098	.....	0.079	0.075	0.081
Oesophagus, length	0.015	.....	0.019	0.021	0.019

The pharynx (*ph*) is relatively smaller, the digestive caeca (*int*) are larger in diameter, and the reproductive fundament (*rf*) is more differentiated. No attempt was made, however, to interpret the various elements of the reproductive complex. A bursa copulatrix is present (not shown in figure). A large, distinct, longitudinally trilobate hold-fast gland (*hfg*) occurs. In the position of the acetabulum and hold-fast organ anterior to the middle of the body, the extensive development of the hold-fast gland and the presence of a bursa copulatrix, *Diplostomulum corti* differs from all other described species of diplostomula. The nervous and excretory systems were not studied. The principal measurements of four mounted specimens are shown in Table II.

Discussion

The oldest known species of the larval group Diplostomulum is *Diplostomulum spathaceum* (Rudolphi), which was described by von Nordmann (1832: 28) as *Diplostomum volvens*. Braun (1894a: 680) showed that according to a feeding experiment performed by the Ehrhardt brothers *Diplostomum volvens* von Nordmann is the larva of *Hemistomum spathaceum* (Rudolphi 1810) Diesing 1850. The name Hemistomum Diesing (1850: 287) falls as a synonym of Alaria Schrank (1788: 52) of which *Alaria alata* (Goeze) is the type species. La Rue (1926a: 15), regarding *Hemistomum spathaceum* (Rudolphi) Diesing as non-congeneric with *Alaria alata,* proposed to make it the type species of a new genus Proalaria. Since, however, *Diplostomum volvens* is the larva of *Hemistomum spathaceum,* it follows that the name of this genus should be Diplostomum von Nordmann [type species, *Diplostomum spathaceum* (Rudolphi)] and the name Proalaria La Rue therefore becomes a synonym. The respective species recognized under the name Proalaria by La Rue (1926b: 277) severally become *Diplostomum spathaceum* (Rudolphi) Olsson, *Diplostomum gavium* (Guberlet) mihi, *Diplostomum indistinctum* (Guberlet) mihi, *Diplostomum confusum*

(Krause) mihi, *Diplostomum excavatum* (Rudolphi) mihi, (?) *Diplostomum intermedium* (Johnston) mihi, (?) *Diplostomum triangulare* (Johnston) mihi and *Diplostomum trilobum* (Rudolphi) mihi. Also *Proalaria huronensis* La Rue (1927: 26) becomes *Diplostomum huronense* (La Rue) mihi. The name Diplostomum, proposed by Brandes (1888: 50) as a new genus (not of von Nordmann, 1832) for certain species of Strigeidae, falls as a homonym.

Now that Diplostomum von Nordmann is a generic name in good standing, it can no longer be used to designate a larval group and it therefore becomes necessary to find another name for that purpose. Brandes (1892: 511), discussing the taxonomy of *Monostomum constrictum* Diesing (1850), made the following statement: "*Monostomum constrictum* Dies., aus dem Auge von Abramis brama, halte ich für eine Form, die zur Unterfamilie der Diplostomen gehört; demnach würde ich die Art als Diplostomulum constrictum bezeichnen." Stiles and Hassal (1908: 156) interpreted this statement to mean that the name Diplostomulum was being proposed as a collective group name and it has since been used in that sense by Faust (1917: 66) and Ward (1918: 411). It is very doubtful that *Monostomum constrictum* is a strigeid larva. It seems desirable, however, to retain Diplostomulum as a group name and to use it for those metacercariae closely resembling *Diplostomum volvens.* Inasmuch as *spathaceum* Rudolphi (1819: 403) has priority over *volvens* von Nordmann (1832: 28), the larva *Diplostomum volvens* von Nordmann becomes *Diplostomulum spathaceum* (Rudolphi) mihi.

It has been the custom of many authors to regard as belonging to a separate larval group (Tylodelphys Diesing, 1850) certain elongate species most of which have all the characters diagnostic of the larval group Diplostomulum (as outlined below), but in which the lateral suckers and the hind-body are not well developed or clearly defined. These differences, in my opinion, are not sufficient to separate these larval groups. The pattern of the reserve bladder of *Tylodelphys clavata* (von Nordmann), the type species, according to von Nord-

mann (1832: Taf. II, fig. 5) and Claparède (1858: Taf. VIII, figs. 2, 3), is not materially different from that of *Diplostomulum browni* and *D. spathaceum*. As a name for the combined group, preference is given to Diplostomulum over Tylodelphys for the reason that the latter may, when the life-history of *Tylodelphys clavata* is known, attain good standing as the generic name of an adult form, since Tylodelphys was not proposed as a larval group name. The species which have been assigned to Tylodelphys are in general more elongate than other diplostomula, since the width of the body is usually less than half the length. They differ also from most diplostomula in that they have ellipsoidal rather than spheroidal calcareous concretions.

The larval group, Diplostomulum, may be redefined as embracing strigeid metacercariae having the following characteristics: (1) fore-body foliaceous, concave ventrally; (2) hind-body present as a small conical prominence on the postero-dorsal part of the fore-body; (3) *reserve bladder* comprised of a system of more or less definitely arranged tubules with calcareous corpuscles, round or ellipsoidal, disposed in vesicles at the termini of small branches; (4) usually a pair of lateral organs (the so-called lateral suckers) on the antero-lateral edges beside the oral sucker; and (5) no true cyst of parasite origin.

Comparative Synopsis of Other Described Diplostomula

1. *Diplostomulum spathaceum* (Rudolphi) mihi

Diplostomum volvens	von Nordmann (1832: 28)
Diplostoma volvens, Nordmann	Cobbold (1860: 50)
Tetracotyle volvens	Mataré (1910: 27)
Diplostomulum volvens	Faust (1917: 66)

Descriptions of *Diplostomulum spathaceum*, based primarily upon the original by von Nordmann (1832: 28), have been published by Creplin (1839: 289), von Nordmann (1843), Diesing (1850: 306, and 1858: 317), Zschokke (1884: 207), Hofer (1904: 294), Lühe (1909: 165), Zandt (1924: 236)

and Plehn (1924: 147). In these descriptions and the reports of Rayer (1843), Dujardin (1845: 474), Creplin (1846: 150, 153), Krøyer (1852–53: 1224), Braun (1892: 796), Olsson (1893: 9) and Fehlmann (1916), *Diplostomulum spathaceum* has been listed from various parts of Europe and from the following fishes: *Scardinius erythrophthalmus, Chondrostoma nasus, Perca fluviatilis, Lucioperca lucioperca, Lucioperca sandra, Acerina cernua, Lota lota, Lota communis, Leuciscus rutilus, Leuciscus vulgaris, Leuciscus phoxinus, Abramis brama, Squalius cephalus, Esox lucius.* The parasites have been found in various parts of the eyes of the hosts infected. For North America Cooper (1915: 191) reported the finding of parasites, which he identified as *Diplostomum volvens,* in the eyes of *Micropterus dolomieu* Lacépède. Steenstrup (1845: 97) suggested that the four forms described by von Nordmann from the eyes of fishes, viz. *Diplostomum clavatum, Holostomum cuticola, Holostomum brevicaudatum* and *Diplostomum volvens,* represent successive stages in the life-cycle of a single species. Claparède (1858: 100; Taf. VIII, figs. 4–5) worked out the relationship of the calcareous corpuscles to the vessels of the reserve bladder. Fraipont (1880: 432) discovered that the flame cells are not associated directly with the vessels of the reserve bladder, a question previously discussed in connection with the excretory system of *Diplostomulum browni.* Braun (1894a, b, and c) reported on feeding experiments performed by the Ehrhardt brothers which demonstrated that *Diplostomulum spathaceum* becomes *Diplostomum spathaceum* in the intestines of *Larus ridibundus* and *Sterna hirundo.* An attempt of the Ehrhardt brothers to cause miracidia of *Diplostomum spathaceum* to penetrate the eyes of goldfish and develop directly (metastatically) into metacercariae was unsuccessful. Roth (1904: 294), however, was of the opinion that such is the manner of the development. Szidat (1924) demonstrated that *Diplostomulum spathaceum* develops from a furcocercous cercaria, that it is therefore digenetic in development, and that the cercaria gains entrance into the host by active penetration through the skin of any

part of the body, seeking the eyes afterward, rather than by direct penetration through the cornea. He also obtained additional evidence in confirmation of the results of the Ehrhardt brothers in regard to the further development of the metacercaria. Salzer (1907) made a careful study of the cataract produced by *D. spathaceum* in the eyes of trout.

A figure by Fraipont (1880: Pl. XVIII, fig. 18) shows that the arrangement of the vessels of the reserve bladder in *Diplostomulum spathaceum* is practically the same as in *Diplostomulum browni*; however, the presence in the former of vesicles containing calcareous corpuscles associated directly with the tubular portions of the urinary bladder constitutes a notable difference. All of several specimens of *Diplostomulum spathaceum* taken from *Cobitis barbatula,* collected near Breslau, Germany, and sent to Professor La Rue by Professor L. Scheuring, are a little larger and relatively much broader than *Diplostomulum browni.* The lateral suckers in these specimens are represented by broad, shallow depressions (Fig. 1), whereas in *D. browni* the lateral organs project slightly (Fig. 5*ls*). These differences in the general shape of the body and the form of the lateral suckers, although constant in the material which I have examined, may be due to the different method of fixation, since the specimens of *D. spathaceum* were fixed in the host tissues in Bouin's mixture, whereas those of *D. browni* were separated from the host tissues and fixed in sublimate-acetic. Although the two forms are morphologically much alike, it seems probable that the occurrence of *D. browni* in a snail host is a physiological character of specific importance, since *D. spathaceum* has never been reported from snails in Europe.

2. *Diplostomulum lenticola* (von Linstow) mihi

Diplostomum lenticola	von Linstow (1878b: 226)
Tetracotyle lenticola von Linstow	Braun (1892: 796)
Tetracotyle lenticola	Mataré (1910: 497)
Tetracotyle lenticola (von Linstow)	Faust (1918: 77)

Descriptions of *Diplostomulum lenticola* based upon the original description of von Linstow (1878b: 226, Taf. VII,

fig. 9) have been published by Lühe (1909: 766) and Faust (1918: 77). This form, which has been listed only from the eyes of *Abramis vimba,* closely resembles *Diplostomulum spathaceum,* differing from it chiefly in that the acetabulum relative to the size of the oral sucker is proportionately smaller. Zandt (1924: 237), however, finds that in the diplostomula from the eyes of various fishes examined by him, the relative sizes of the oral sucker and acetabulum vary greatly in different individuals and considers that they all probably belong to the same species. Whether *Diplostomulum lenticola* is a distinct species is, therefore, a matter that requires further investigation.

3. *Diplostomulum petromyzi-fluviatilis* (Diesing) mihi

Entozoa from *Petromyzon fluviatilis*	Müller (1840: 198)
Diplostomum rachineum	Müller (1842: 20)
Diplostomum petromyzi fluviatilis Müller	Diesing (1850: 307)
(?) *Diplostomum* sp. *in Petromyzon fluviatilis*	Krøyer (1852–53: 1051)
Tylodelphys (?) *Petromyzonis fluviatilis*	Diesing (1858: 316)
Diplostoma, Mülleri, Cobbold	Cobbold (1860: 50)
Entozoa in *Petromyzon planeri*	Gulliver (1870: 849)
Neuronaia lampetrae	Gulliver (1872: 103)
Tylodelphys (?) *Petromyci fluviatilis* Diesing	von Linstow (1878a: 290)
Neuronaina Lampretae Gulliver	von Linstow (1878a: 290)
Tetracotyle Petromyzontis	Brown (1899: 489)
Tylodelphys petromyzi fluviatilis Diesing	Lühe (1909: 168)
Tetracotyle petromyzi	Mataré (1910: 496)

This species, found and reported by Müller (1840: 198) and briefly described by Gulliver (1870: 849; fig. 9), was carefully studied by Brown (1899), from whose description others have been published by Lühe (1909: 168) and Faust (1918: 77). It has been reported from the cranial and brain cavities of both *Petromyzon fluviatilis* and *P. planeri* in Europe. According to Brown (1899, Pl. 39, fig. 5), many

gland cells are associated with the lateral suckers, a feature which has not been reported for other species of Diplostomulum. In preserved specimens sent to Professor La Rue by Professor E. S. Goodrich, of Oxford, the lateral suckers are more deeply cup-shaped (Fig. 2) than in any other described species of diplostomula; and when everted they become, accordingly, more prominent (Fig. 4). In *D. browni* (Fig. 8) and *D. spathaceum* the concavity of the ventral surface is lost and the fore-body is somewhat thickened in the process of fixation. Sections of *D. petromyzi-fluviatilis* show the ventral surface to be distinctly concave and the fore-body relatively thin.

Inasmuch as a satisfactory series of measurements of *Diplostomulum petromyzi-fluviatilis* has never been recorded, those which I have made of ten specimens mounted *in toto* are presented in Table III.

TABLE III

Measurements in Millimeters of Ten Mounted Specimens of Diplostomulum petromyzi-fluviatilis

	Minimum	Maximum	Average
Body			
length	0.225	0.289	0.257
width	0.211	0.261	0.239
Oral sucker			
length	0.042	0.053	0.050
width	0.042	0.053	0.046
Pharynx			
length	0.031	0.049	0.041
width	0.017	0.025	0.021
Acetabulum			
length	0.031	0.049	0.038
width	0.053	0.060	0.056
Hold-fast organ			
length	0.063	0.109	0.076
width	0.105	0.112	0.106
Cavity of lateral sucker,			
depth	0.024	0.035	0.030

4. *Diplostomulum phoxini* (Faust) mihi

Tetracotyle from brain of *Phoxinus laevis*	Mataré (1910)
Tetracotyle phoxini	Faust (1918: 77)
Tylodelphys from *Phoxinus laevis*	André (1918: 13)
Tetracotyle phoxini Mataré	Mathias (1925: 17)
Tetrocotyle phoxini (Faust)	Ashworth and Bannerman (1927: 167)

This species was described in detail by Mataré (1910) and briefly by André (1918), the descriptions being based upon specimens from the brain and cranial cavity of *Phoxinus laevis,* from different parts of Switzerland. The name *Tetracotyle phoxini* was applied to Mataré's description by Faust (1918: 77). Mataré's specimens, measured apparently in life, averaged 0.2 mm. in length and 0.15 mm. in width. Those of André, preserved and sectioned, were, on the average, 0.23 mm. long and 0.15 mm. wide. Ashworth and Bannerman (1927) described in detail a Diplostomulum found in the brain and cranial cavity of *Phoxinus phoxinus* from near Edinburgh. Although their discoveries in regard to minutiae of the excretory and nervous systems differ somewhat from the findings of Mataré and their specimens were a little larger (from 0.27 mm. to 0.42 mm. in length, in life), they were of the opinion that their material was identical with *Tetracotyle phoxini* Faust. Ten of several preserved diplostomula, taken from the brain and cranial cavity of *Phoxinus laevis* from Lunz and presented to Professor La Rue by Professor L. Scheuring, of München, varied in length from 0.203 mm. to 0.276 mm. (average 0.219 mm.) and in width from 0.109 mm. to 0.131 mm. (average 0.121 mm.). These specimens are probably identical with Mataré's species, and their measurements, allowing for shrinkage in the process of fixation, correspond favorably with those of Ashworth and Bannerman. According to both Mataré (1910: 510) and Ashworth and Bannerman (1927: 163), the cuticula is entirely without spines, an unusual circumstance for strigeid larvae. The calcareous concretions are more or less spherical (Ashworth and Bannerman, 1927: Figs. 5–6). In most of our specimens the lateral suckers have the form shown in Fig. 3.

5. *Diplostomulum heterobranchi* (Wedl) mihi

Monocerca heterobranchi Wedl (1861: 478)
Tetracotyle heterobranchi Mataré (1910: 497)

This parasite, found in thousands in adipose tissue about the brain of *Heterobranchus anguillaris* in Egypt, was described briefly by Wedl (1861: 478; Taf. III, fig. 39). According to the figure, the body of the parasite is elongate like that of a Tylodelphys; the calcareous concretions, however, are spheroidal. The presence of a hind-body was not indicated.

6. *Diplostomulum putorii* (von Linstow) mihi

Diplostomum putorii von Linstow (1877: 191)
Tetrocotyle putorii Mataré (1910: 497)

This worm, found encysted in the gut and oesophagus of *Foetorius putorius,* was briefly described by von Linstow (1877: 191; Taf. XIV, fig. 21). According to the figure, the animal is pointed anteriorly and without lateral suckers; the calcareous concretions are spheroidal and the pharynx is larger than the oral sucker. This is the only Diplostomulum recorded from a mammal. The presence of a hind-body was not indicated.

7. *Diplostomulum odhneri* (Travassos) mihi

Larva of a Prohemistomum Travassos (1924: 836)

This form was found encysted beneath the skin of *Haemulon sp.* in Brazil and briefly described by Travassos (1924: 836; fig. 1). According to the figure, the animal is without lateral organs and a hind-body. The acetabulum is somewhat smaller than the oral sucker. Nothing is said concerning the excretory system. No fundament of reproductive organs could be found. By a feeding experiment the larvae were developed into *Prohemistomum odhneri* Travassos in the intestines of *Nyctanassa violacea* L.

8. *Diplostomulum nanus* (Stiles and Goldberger) mihi

Agamodistomum nanus Stiles and Goldberger (1908: 30)

This species was described by Stiles and Goldberger (1908) from specimens taken from the pectoral muscles of *Francolinus*

subtorquatus from Benguella, West Africa. Through the courtesy of Dr. C. W. Stiles, of Washington, D. C., to Professor La Rue, I have had an opportunity to examine type and cotype material of *Agamodistomum nanus*. The structure referred to by Stiles and Goldberger (1908, fig. 45) as a "genital pore" is the external opening of the cavity of a hold-fast organ and the anterior "primordium of genital glands" (fig. 52) is the hold-fast gland. The species is in reality, therefore, a Diplostomulum.

9. *Diplostomulum vegrandis* (La Rue) mihi

Cercaria vegrandis La Rue (1917: 8)

This species was found encysted in the fat bodies of *Thamnophis marciana* (Baird and Girard) and *Thamnophis eques* (Reuss) from western Texas and described by La Rue (1917: 8; Pl. 1, figs. 2, 3, 4). The description is based upon preserved material and it was not possible to study the details of the excretory system and the shape of the calcareous concretions. The round or oval cavities found in the parenchyma probably represent vacuities in which lay calcareous corpuscles. Lateral suckers are not present and a hind-body was not observed. Through the courtesy of Professor La Rue I have been able to examine type and paratype material of this species. The structure described as "probably the genital pore" is clearly homologous to the hold-fast opening in strigeid larvae. The species is, therefore, a Diplostomulum.

10. *Diplostomulum sirtali* (Nicoll) mihi

Diplostomum sirtali Nicoll (1912: 767)

This parasite, encysted in the mesenteric fat of three-striped snakes, *Tropidonotus ordinatus sirtalis* [*Thamnophis sirtalis sirtalis* (Linné)], was found and described by Nicoll (1912: 769; fig. 107). These snakes, originally from North America, died in the Gardens of the Zoological Society of London. The conditions under which the snakes had been kept were not recorded and it is not possible to determine whether

they acquired their infections in England or America. Since, however, a similar parasite is not known to occur in European snakes, it seems probable that it is an American form. *D. sirtali* is somewhat similar to *D. vegrandis,* from which it differs in that it is rather larger and that the fore-body is distinctly concave ventrally, the hind-body is plainly visible and the acetabulum is slightly anterior to the middle of the body—differences which may be due, at least in part, to the fact that *D. sirtali* was described in life, whereas *D. vegrandis* was described from preserved material. Although the arrangement of excretory vessels was not studied, the presence of calcareous corpuscles distributed throughout the body was observed.

11. *Diplostomulum rhachiaeum* (Henle) mihi

Parasite from *Rana esculenta* and *R. temporaria*	Caldani (1794: 312)
Vermes generis dubii ranarum	Rudolphi (1810: 268)
Diplostomum rhachiaeum	Henle (1833: 19)
Diplostomum rachiaeum	Dujardin (1845: 475)
Tylodelphys rhachidis Diesing	Diesing (1850: 305)
Tylodelphis rhachidis	Pagenstecher (1857a: 38)
Diplostoma rhachiaeum, Henle	Cobbold (1860: 49)
Diplostomum rachieum	Fraipont (1880: 419)
Diplostomum (*Tylodelphis*). *rachidis*	Parona (1896: 2)
Tylodelphys rhachiaea (Henle)	Lühe (1909: 168)
Tetracotyle rhachiaea	Mataré (1910: 496)

This species, found encysted in the vertebral canal and in the viscera of *Rana esculenta* and *R. temporaria,* described and figured, but not named, by Caldani (1794: 312; Pl. 7, figs. 7, 8), is perhaps the oldest known strigeid metacercaria. Not aware of Caldani's report, Henle (1833) again described the species as new. Other descriptions based on that of Henle have been published by Dujardin (1845: 475), Diesing (1850: 305), Pagenstecher (1857: 38) and Lühe (1909: 168). The measurements recorded by Pagenstecher were original. The association of the ovoidal calcareous corpuscles with the excretory tubules was shown by Claparède (1858: 99; Taf. VIII, figs. 1, 2, 3). The body is elongate, its length being more than

twice its width. The lateral suckers are visible as ear-like processes only in certain contraction states (Henle, 1833, fig. 19). The hind-body is not clearly set off from the fore-body.

12. *Diplostomulum clavatum* (von Nordmann) mihi

Diplostomum clavatum	von Nordmann (1832: 42)
Tylodelphys clavata Diesing	Diesing (1850: 305)
Diplostoma clavatum, Nordmann	Cobbold (1860: 49)
Tetracotyle clavata	Mataré (1910: 496)

Descriptions of this species based on the original by von Nordmann (1832: 42) have been published by von Nordmann (1843), Dujardin (1845: 475), Diesing (1850: 305) and Lühe (1909: 168). In these descriptions and the reports of Krøyer (1846–53: 253) and Zandt (1924: 241), it has been listed from the eyes of the following hosts: *Perca fluviatilis, Lucioperca lucioperca, Acerina cernua, Esox lucius, Abramis brama, Barbus fluviatilis, Squalius cephalus, Leuciscus leuciscus, Leuciscus rutilus,* and *Salmo salvelinus.*

Diplostomulum clavatum closely resembles *D. rhachiaeum* in regard to the general shape of the body, the shape and the arrangement of the calcareous corpuscles, the structure of the lateral suckers and the condition of the hind-body.

13. *Diplostomulum craniarium* (Diesing) mihi

Trematodum from cranial cavity of *Cobitis fossilis*	Leydig (1853: 382)
Tylodelphys (*?*) *craniaria*	Diesing (1858: 316)
Diplostoma craniarium, Cobbold	Cobbold (1860: 49)
Tylodelphis craniaria Dies.	Braun (1892: 796)
Tylodelphys craniaria	Pavesi (1881: 616)
Tylodelphys craniaria	Mataré (1910: 496)

This species was found in the cranial cavity of *Cobitis fossilis,* described and figured, but not named, by Leydig (1853: 382; Taf. XIV, fig. 6). Leydig confused the anterior and posterior regions of the body, and the digestive and excretory systems. His figure, however, shows the body to be elongate and the calcareous corpuscles ovoidal. Brief descriptions based on that of Leydig were published by Diesing (1850:

316) and Lühe (1909: 168). The occurrence of the parasite has been reported only once.

14. *Diplostomulum joyeuxi* mihi

Metacercaria (*Diplostomum*) sp. Joyeux (1923: 334)

This parasite, described and figured by Joyeux (1923: 334; fig. 3), occurs encysted in the muscles of *Rana esculenta ridibunda* Pallas in Tunis, Africa. As represented in the figure, lateral suckers and hind-body are apparently wanting; the development of the reproductive system is well advanced, with testes, ovary, uterus, genital pore and vitellaria differentiated; the excretory system is very simple, consisting of "un collier entourant le pharynx, deux troncs longitudinaux decendant le long du corps et se réunissant à l'extrémité postérieure pour former un pore excréteur." The author does not state whether observations were made on living material. If only preserved material was studied, it is possible that the "collier entourant le pharynx" pertains to the nervous system and that the nervous and excretory systems were, in part, confused. This assumption is supported by the fact that in preserved specimens, mounted *in toto*, of *Diplostomulum browni*, *D. spathaceum*, and *D. petromyzi-fluviatilis*, the posterior ends of posterior nerve trunks are superimposed upon the tubular portions of the urinary bladder, whereas other parts of the excretory system are not recognizable. The presence of calcareous corpuscles was not mentioned.

Species Inquirendae

Inasmuch as it is doubtful that the following species belong to the larval group Diplostomulum, they are listed under their oldest known names.

1. *Diplostomum parvulum* Stafford

Tetracotyle parvula Mataré (1910: 497)
Diplostomulum parvulum Faust (1917: 66)

The type specimens of this species were taken from cysts about the pharynx of *Semotilus bullaris* Rafinesque. The

description (Stafford, 1904: 494) is not complete enough to permit one to decide whether it has to do with a species of Diplostomulum or Tetracotyle. No figure has been published.

2. *Diplostomum cobitidis* von Linstow

Tetracotyle cobitidis Mataré (1910: 497)

This species was described by von Linstow (1890: 179) from specimens found in thick-walled cysts in the body cavity of *Cobitis barbatula.* Here, again, no figure is given and the description is too brief to show whether the parasite is a Diplostomulum or a Tetracotyle. Zandt (1924: 239) reported the finding of *D. cobitidis* in *Cobitis barbatula* and *Cottus gobio* in the Bodensee and stated that, according to the key of Lühe, the parasite was probably a Tetracotyle. I have examined specimens from *Cottus gobio* which were designated by Dr. Zandt, of Konstanz, as *Diplostomum cobitidis* and presented to Professor La Rue. I find these worms to be tetracotyles, perhaps identical with *Tetracotyle ovata* von Linstow.

3. *Diplostomum* sp. Zandt

The description of this form is based on a single specimen found in the vitreous humor of *Coregonus fera* (Zandt, 1924, p. 240; Fig. 14) from the Bodensee, Konstanz. According to the figure, the worm has the form of *Diplostomulum spathaceum* with the body deeply constricted near the middle, anterior to the acetabulum. Otherwise in form and size it closely resembles *D. spathaceum.* It seems probable that the constriction is an artifact, due possibly to the presence of a girdle of connective tissue about the body. I have sometimes seen diplostomula, taken from the lenses of American fishes, with the body constricted in a similar manner, when they were in the act of passing through the lens capsule. So long as the lens and the capsule remain intact, a parasite can work its way through with ease if it once perforates the lens epithelium. If, however, a piece of the lens epithelium, containing a diplostomulum which has worked half through a pore in this epithelium, should be torn from the lens, the parasite

could no longer alter its position relative to that of the tissue about it. Lens epithelium is very delicate and a tiny strand of it embedded, as it were, in the infolded cuticula of the parasite would be hardly visible.

4. *Distomum coelebs* von Linstow

Agamodistomum coelebs Linstow — Stossich (1892: 176)

This worm was found encysted in the wall of the intestine of *Fringilla coelebs* and briefly described by von Linstow (1875: 192; Taf. III, fig. 15). Mataré (1910: 496) suggested that *Distomum coelebs* might be a larval strigeid. This supposition is supported by the fact that a large, bright, spherical structure, possibly a hold-fast organ, is located behind the acetabulum. The large size of the spines on the anterior part of the body and the brevity of the broad intestinal caeca are, however, characters not common to strigeid metacercariae. The finding of the species has been reported only once.

5. *Tetracotyle ardeae* Mataré

Geschlechtlich unentwickeltes Distoma — Wedl (1857: 247)
Distomum capsulare — Diesing (1858: 355)
Agamodistomum capsulare Diesing — Stossich (1892: 34)

This parasite was found encysted in the oesophagus, pectoral muscles, and under the skin of the head and neck of *Ardea purpurea, Ardea nycticorax, Ardea cinerea, Gallinula crex* and *Podiceps negricollis* and described by Wedl (1857: 247; Taf. I, fig. 8) as a sexually undeveloped distome. Mataré (1910: 496) regarded it as a strigeid metacercaria. According to the figure, the presence of a hind-body, a hold-fast organ, or calcareous corpuscles is not evident. The species is probably not a strigeid metacercaria. Pagenstecher (1857b: 246) found, but did not describe, a species of Tetracotyle encysted in the neck muscles of a wild duck.

6. *Monostomum constrictum* Diesing

Diplostomulum constrictum Dies. — Brandes (1892: 511)

This form was very briefly described by Diesing (1850: 322, and 1855: 62; Taf. II, figs. 3–5) from trematodes found

in the eyes of *Abramis brama*. According to the figures, the animal is apparently without an acetabulum and a hold-fast organ. The fact that the body is divided into distinct anterior and posterior regions by a constriction is hardly sufficient evidence to warrant the conclusion of Brandes (1892: 511) that the worm is a strigeid larva. Lühe (1909: 168) regarded it as a Tylodelphys. If *Monostomum constrictum* is a strigeid metacercaria at all, it probably belongs to the larval group Neascus, a conclusion suggested by the arrangement of the excretory vessels (Diesing, 1855; Taf. II, fig. 5).

7. *Distomum retroconstrictum* Srámek

The parasite described and figured by Srámek (1901: 108; Fig. 9) as *Distomum retroconstrictum* and regarded as identical with *Monostomum constrictum* Diesing is apparently a specimen of *Diplostomulum clavatum* with an accidental constriction near the anterior end. Srámek confused the anterior and posterior ends of the animal and misinterpreted the hold-fast organ for the mouth, and the mouth for the excretory pore.

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I wish here to tender my sincere appreciation to George R. La Rue, professor in the Zoology Department and director of the Biological Station, University of Michigan, by whom this study was suggested and under whom it has been conducted.

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For aid in securing material for study my thanks are due to the following: Professor L. Scheuring of the Bayerische Biologische Versuchsanstalt für Fischerei, München; Professor Ferdinand Zandt of the Anstalt für Bodensee-forschung der Stadt Konstanz; Professor E. S. Goodrich of the Department of Zoology and Comparative Anatomy, University

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LITERATURE CITED

André, A. 1918. Notes de parasitologie. Bull. Soc. Vaudois Sci. Nat., (5) 52: 12–14.

Ashworth, A. W., and Bannerman, J. C. W. 1927. On a Tetracotyle (T. phoxini) in the Brain of the Minnow. Trans. Roy. Soc. Edinburgh, 55: 159–172, 1 pl., 8 text figs.

Brandes, G. 1888. Die Familie der Holostomeae. Ein Prodromus zu einer Monographie derselben. 72 pp. Leipzig.

——. 1892. Revision der Monostomiden. Centralbl. f. Backt. u. Parasitenk., 12: 504–511.

Braun, M. 1892. Vermes. In Bronn's Kl. u. Ord. d. Thier-Reichs. 4, Abt. IA. 925 pp. Leipzig.

——. 1894a. Helminthologische Notizen. IV. Zur Entwicklungsgeschichte der Holostomiden. Centralbl. f. Backt. u. Parasitenk., 15: 680–682.

——. 1894b. Zur Entwicklungsgeschichte der Holostomiden. Nach den Untersuchungen der Herren Alfred und Oscar Ehrhardt mitgetheilt. Zoöl. Anz., 17: 165–167.

——. 1894c. Abstract of the preceding paper. Zoöl. Zentralbl., 1: 755.

Brown, A. W. 1899. On *Tetracotyle petromyzontis*, a Parasite of the Brain of Ammocoetes. Quart. Journ. Microsc. Soc. N. S., 41: 489–498, 1 pl.

Caldani, F. 1794. Osservazioni sopra la transformazione di un insetto e sopra le idatide della ranocche. Mem. Soc. Ital., Verona, 7:305–318, 2 figs.

Claparède, E. 1858. Ueber die Kalkkörperchen der Trematoden und die Gattung Tetracotyle. Zeitschr. f. wissensch. Zool. 9: 99–105, 1 pl.

Cobbold, T. S. 1860. Synopsis of the Distomidae. Journ. Proc. Linn. Soc. Lond., 5: 1–56.

Cooper, A. R. Trematodes from Marine and Fresh-water Fishes. Trans. Roy. Soc. Canada, (3) 9: 181–205, 3 pls.

Creplin, F. C. H. 1839. Eingeweidewürmer, Binnenwürmer, Thierwürmer. Allg. Encycl. d. Wissensch. u. Künste (Ersch und Grube), 1 (32): 277–302.

——. 1846. Nachträge zu Gurlt's Verzeichniss der Thiere, bei welchen Entozoen gefunden worden sind. Arch. f. Naturg., 12: 129–160.

Diesing, C. M. 1850. Systema Helminthum, Vol. 1, 679 pp. Vindobonae.

——. 1855. Neunzehn Arten von Trematoden. Denkschr. d. k. Akad. d. Wissensch. Math.-nat. Kl., 10: 59–70, 3 pls.

——. 1858. Revision der Myzhelminthen. Abt. Trematoden. Sitzungsb. d. k. Akad. d. Wissensch. Math.-nat. Kl., 32 (23): 207–390, 2 pls.

Dujardin, F. 1845. Histoire naturelle des helminthes ou vers intestinaux. 654 pp. Paris.

Faust, E. C. 1917. Life History Studies on Montana Trematodes. Ill. Biol. Monogr., 4 (1): 7–120, 9 pls.

——. 1918. The Anatomy of *Tetracotyle iturbei*, with a Synopsis of Described Tetracotyliform Larvae. Journ. Parasitol., 5: 69–79, 1 pl.

Fehlmann, J. W. 1916. Eine unangenehme Beobachtung. Schweiz. Fisch.-Zeitz., 23: 68–69.

Fraipont, J. 1880. Recherches sur l'appareil excrétur des trematodes et des cestodes. Arch. de Biol., 1: 415–456, 2 pls.

Gulliver, G. 1870. On Certain Points in the Anatomy and Economy of the Lampreys. Proc. Zool. Soc. London, for 1870: 844–850, 9 figs.

——. 1872. (Note without title.) Quart. Journ. Microsc. Soc., N. S., 12: 102–103.

Henle, F. G. J. 1833. Ueber das *Diplostomum rhachiaeum*, einen Eingeweidewurm der Wirhelhöhle. Notiz. a. d. Geb. d. Nat. u. Heilk., 38: 19–22.

Hofer, B. 1904. Handbuch der Fischkrankheiten. 359 pp. München.

Joyeux, C. 1923. Recherches sur la faune helminthologique africaine. Archives de l'Institut Pasteur de Tunis, 12: 328–338, 4 figs.

Krøyer, H. 1846–53. Danmark's Fiske. Vol. 3 (1 Afd.). 704 pp. Kjøbenhavn.

——. 1852–53. Ibid., Vol. 3 (2 Afd.): 705–1279. Kjøbenhavn.

La Rue, G. R. Two New Larval Trematodes from *Thamnophis marciana* and *Thamnophis eques*. Occasional Papers Mus. Zool. Univ. Mich. No. 35; 12 pp. 1 pl.

——. 1926a. Studies on the Trematode Family Strigeidae (Holostomidae) No. II. Taxonomy. Trans. Amer. Microscop. Soc., 45: 11–19.

——. 1926b. Studies on the Trematode Family Strigeidae (Holostomidae) No. III. Relationships. Ibid., 45: 265–281, 1 pl.

——. 1927. Studies on the Trematode Family Strigeidae (Holostomidae) No. V. *Proalaria huronensis*, sp. nov. Ibid., 46: 26–35, 2 pls.

Leydig, F. 1853. Zoologische Notizen. 2 Helminthologisches. Zeitschr. f. wissensch. Zool., 4: 382–387, 3 figs.

Linstow, O. F. B. von. 1875. Beobachtungen an neuen und bekannten Helminthen. Arch. f. Naturg., 41: 183–207, 3 pls.

——. 1877. Enthelminthologica. Ibid., 43: 173–198, 3 pls.

——. 1878a. Compendium der Helminthologie. 382 pp. Hannover.

——. 1878b. Neue Beobachtungen an Helminthen. Arch. f. Naturg., 44: 218–245, 3 pls.

——. 1890. Beitrag zur Kenntniss der Vogeltänian, nebst Bermerkungen über neue und bekannte Helminthen, Ibid., 56: 171–188, 1 pl.

Lühe, M. 1909. Parasitische Plattwürmer I. Trematoden. Brauer's Süsswasserfauna Deutschlands, Heft 17. 217 pp.

Mataré, F. 1910. Ueber eine neue Tetracotyle im Hirn von *Phoxinus laevis*. Zeitschr. f. wissensch. Zool., 94: 488–540, 1 pl., 3 text figs.

Mathias, P. 1925. Recherches expérimental sur le cycle évolutif de quelques trématodes. Bull. Biol. Fr. Bel., 59: 1–124, 4 pls., 13 figs.

Müller, J. 1840. Vergleichende Neurologie der Myxinoiden. Abhand. d. k. Akad. d. Wissensch. zu Berlin, 1838: 171–251, 4 pls.

——. 1842. On *Diplostomum rachineum* (Abstract of 1840). Microscop. Journ. and Structural Record, 2: 20–21.

Nicoll, W. 1912. On Two New Larval Trematodes from the Striped Snake (*Tropidonotus ordinatus sirtalis*). Proc. Zool. Soc. London (1912): 767–770, 1 fig.

Nordmann, A. von. 1832. Mikrographische Beiträge zur Naturgeschichte der wirbellosen Thiere. I. 118 pp. Berlin.

——. 1843. Sur les Helminthes dans l'oeil des animaux supérieurs. Arch. de Méd. Comparée, 1: 67–113.

Olsson, P. 1893. Bidrag till skandinaviens helminthfauna. 2. K. Svenska Vetensk. Akad. Handl., N. F., Vol. 25 (2) Art. 12, 41 pp., 5 pls.

Pagenstecher, H. A. 1875a. Trematodenlarven und Trematoden. Helminthologischer Beitrag. 56 pp., 6 pls. Heidelberg.

——. 1875b. Ueber Erziehung des *Distoma echinatum* durch Fütterung. Arch. f. Naturg., 23 (1): 244–251.

Parona, C. 1896. Helminthum ex Conradi Paronae Museo Catalogus. 44 pp. Genova.

Pavesi, P. 1881. Dalle mie annotazioni zoologische. Trematode nuovo parasita d'un pesce fluviale. R. Ist. Lomb. di. Sci. e. Lett., 2. s.) 14: 615–616.

Plehn, M. 1924. Praktikum der Fischkrankheiten. 179 pp. Stuttgart.

Rayer, P. 1843. Note sur les Helminthes des yeux. Arch. de Méd. Comparée, 1: 113–154.

Roth, W. 1904. Beiträge zur Kenntniss der parasitären Fischkrankheiten. V. Uber der grauen Star bei Aquariumfischen. Natur und Haus, 13 (3): 41–44, 2 figs.

Rudolphi, C. A. 1810. Entozoorum sive Vermium Intestinalium Historia Naturalis. Vol. 2. 386 pp. Amstelaedami.

——. 1819. Entozoorum Synopsis. 811 pp., 3 pls. Berolini.

Salzer, F. 1907. Anatomische Untersuchungen über den Wurmstar der Fische. Archiv. f. Augenheilk., 58: 19–39, 7 figs.

Schrank, F. v. P. 1788. Verzeichniss der bisher hinlänglich bekannten Eingeweidewürmer nebst einer Abhandlung über ihre Anverwandtschaften. 116 pp., 1 pl. München.

Srámek, A. 1901. Helminthen der an der zoologischen Station in Podiebrad (Böhmen) untersuchten Fische. Arch. f. d. Naturw. Landesdurchforsch. v. Böhmen, 11 (3): 94–118, 22 figs.

Stafford, J. 1904. Trematodes from Canadian Fishes. Zool. Anz. 27: 481–495.

Steenstrup, J. J. S. 1845. On the Alternation of Generations; or, The Propagation and Development of Animals through Alternate Generations: a peculiar form of fostering the young in the lower classes of animals [translated from the German version of C. H. Lorenzen, by George Busk]. 132 pp. London.

Stiles, C. W., and Goldberger, J. 1908. Observations on Two New Parasitic Trematode Worms: *Homalogaster philippinensis* n. sp., *Agamodistomum nanus* n. sp. Hyg. Lab. Bull. No. 40: 23–33, 14 pls.

Stiles, C. W., and Hassall, A. 1908. Index Catalogue of Medical and Veterinary Zoology. Trematoda and Trematode Diseases. Hyg. Lab. Bull. No. 37: 401 pp. Washington.

Stossich, M. 1892. I distomi degli uccelli. Biol. Soc. Adriat. Sci. Nat., Trieste, 13: 143–196.

Szidat, L. 1924. Beiträge zur Entwicklungsgeschichte der Holostomiden. Zool. Anz., 61: 249–266, 12 figs.

Travassos, L. 1924. Nouvelle espèce de Prohemistomum. C. R. Soc. Biol., 91: 835–838, 3 figs.

Ward, H. B. 1918. Parasitic Flatworms. In Ward and Whipple's Fresh-water Biology. Chap. XIII: 265–453. New York.

Wedl, K. 1857. Anatomische Beobachtungen über Trematoden. Sitzungsb. d. K. Akad. d. Wissensch. Math.-Nat. Kl., 26: 241–278, 4 pls.

——. 1861. Zur Helminthenfauna Ägyptens. 2 Abt. Ibid., 44 (1 Abt.): 463–482, 3 pls.

Zandt, F. 1924. Fischparasiten des Bodensees. Centralbl. f. Backt. u. Parasitenk. (I. Abt. Orig.), 92:225–271, 33 figs.

Zschokke, F. 1884. Recherches sur l'organisation et la distribution zoologique des vers parasites des poissons d'eau douce. Arch. de Biol., 5: 153–241, 2 pls.

Explanation of Figures

ac —acetabulum
acv —anterior transverse commissural excretory vessel
al —anterior lateral excretory vessel
an —anterior nerve
apt —anterior primary collecting tubule
c —cuticula
cc —calcareous concretion
cmf —circular muscle fibers
ep —excretory pore
ed —excretory duct
gc —unicellular gland cell
hb —hind-body
hf —hold-fast organ
hfg —hold-fast gland
int —intestinal caecum
j —apparent point of union of primary excretory apparatus with reserve bladder
lc —lateral collecting vessel
lmf —longitudinal muscle fibers
ls —lateral sucker
md —median dorsal excretory vessel
oe —oesophagus
os —oral sucker
pc —posterior transverse commissural excretory vessel
ph —pharynx
pl —posterior lateral excretory vessel
pn —posterior nerve
ppt —posterior primary collecting tubule
rf —fundament of reproductive organs
sp —supra-pharyngeal nerve commissure
tb —tubular portion of urinary bladder
ub —urinary bladder

PLATE I

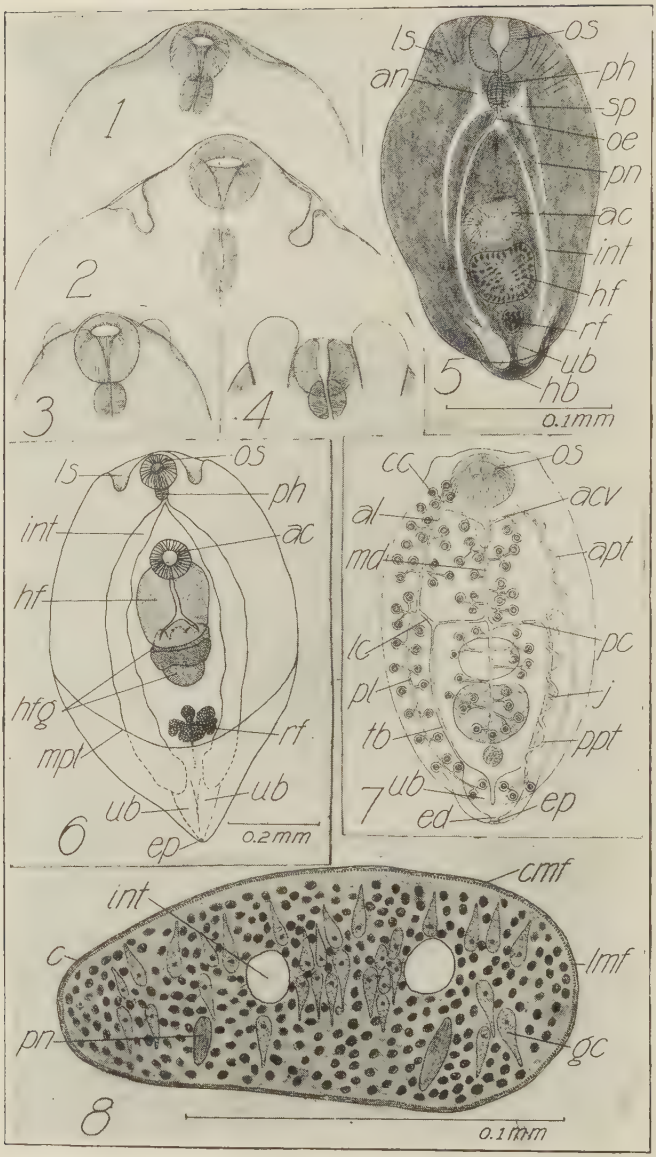
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PLATE I

Figures 1, 2, 3, 4 and 5 were drawn from specimens mounted *in toto* with the aid of a camera lucida and to the same magnification.

FIG. 1. *Diplostomulum spathaceum,* ventral view of the anterior end showing the lateral suckers as broad shallow depressions, the form in which they usually appear in preserved material

FIG. 2. *Diplostomulum petromyzi-fluviatilis,* ventral view of the anterior end showing the usual form of the lateral suckers in preserved specimens

FIG. 3. *Diplostomulum phoxini,* ventral view of the anterior end showing the usual form of the lateral suckers in preserved worms

FIG. 4. *Diplostomulum petromyzi-fluviatilis,* dorsal view of the anterior end of a specimen having the body much contracted laterally and the lateral suckers everted. This is an unusual condition in preserved specimens

FIG. 5. *Diplostomulum browni,* ventral view showing the salient morphological features and the lateral suckers as they usually appear in preserved specimens

FIG. 6. *Diplostomulum corti,* ventral view showing the principal internal structures. From a sketch made by Dr. W. W. Cort

FIG. 7. *Diplostomulum browni,* a semi-diagrammatic representation of the excretory system in dorsal view. Based upon a study of living specimens much flattened by cover-glass pressure

FIG. 8. *Diplostomulum browni,* cross-section through the fore-body just anterior to the acetabulum

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) No. XVII: *TETRACOTYLE FLABELLIFORMIS* FAUST*

R. CHESTER HUGHES

THE parasites upon which this study has been made differ in several important respects from *Cercaria flabelliformis* as originally described (Faust, 1917 a, p. 111, Fig. 4). It seems probable, however, for reasons which are discussed later, that the two forms are identical. The structural differences found in my studies, as compared with the descriptions by Faust, are of sufficient magnitude to warrant a redescription of the species.

MATERIAL AND METHODS

The material upon which this study is based consists of a cotype specimen of *Cercaria flabelliformis* Faust, stained and mounted *in toto*, and a collection of tetracotyles taken by me from snails at Douglas Lake, Cheboygan County, Michigan, during the summer of 1927. The cotype specimen was submitted by Faust with his doctor's thesis to the Department of Zoölogy of the University of Illinois, and, through the courtesy of Professor Henry B. Ward to Professor La Rue, it has been made available to me.

An examination of 13 specimens of *Lymnaea stagnalis perampla* Walker collected from Douglas Lake showed 9 to be infected with from 5 to 765 (average 118) tetracotyles per snail. A single specimen of *Planorbis campanulatus* Say contained 23 parasites of apparently the same species. Alcoholic specimens taken from each

* Contribution from the Biological Station and from the Department of Zoölogy, University of Michigan. This is the seventeenth of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

of these hosts have been deposited as lots numbered 213 and 203, respectively, in the parasite collection of the Museum of Zoölogy of the University of Michigan. In every specimen the worms occurred scattered through the soft parts (liver and reproductive gland) of the host. In *Planorbis* several specimens were found in the sporocysts of other trematodes, a condition not observed in *Lymnaea*, although the latter sometimes harbored parthenitae. In general the specimens taken from *Planorbis* have the hind-body a little broader and less distinctly set off from the fore-body, differences, however, which are seemingly too slight to warrant the establishment of a new species for them. In the type host, *Physa gyrina* Say from Corvallis, Montana, the parasite was sometimes found in the rediae of other trematodes (Faust, 1917 a, p. 111).

The method employed for the fixation of material and the preparation of slides is the same as that used in my study of *Tetracotyle pipientis* Faust (Hughes, 1928, p. 43). Here again the same advantages were found in the study of living material. The cotype specimen was treated with the same stain as that used in my preparations.

THE CYST

During the early part of its development the tetracotyle is free in the tissues of the host. The more mature individuals, however, are always encysted. The nature of the cyst is very peculiar and deserves special consideration. In *Tetracotyle cornuta* (Rudolphi) (*T. typica* Diesing), a closely related European species, the cyst has long been a subject of controversy. In living material of the present form the cyst consists of a thick layer of gelatinous substance closely adherent to the cuticle of the parasite and with perforations adjacent to the excretory pore, and the external openings of the oral sucker, lateral suckers and the cavity of the hold-fast organ. Steenstrup (1845, p. 80) stated that when fully grown the Distomata (tetracotyles) "are covered by a very thick cuticle, appearing as if they were enveloped in a thick layer of inspissated albumen." Pagenstecher (1857, p. 32) spoke of the cyst as follows: "Trotz ihrer doppelten Umrisse kann man sie eigentlich nicht als Cysten bezeichnen, denn der zweite Umriss wird allein

von der abgelösten alten Haut des Thieres gebildet." Ercolani (1881, p. 287) wrote: "Che la grossa e diafana membrana che avvolge tutto l'animale, non sia una cisti, ma una dipendenza della pelle, è attestato dal fatto che essa aderisce non solo tenacemente su tutta la superficie del corpo dell' animale, ma che in corrispondenza del poro terminale deve rimanere aperta se lascia sfuggire i materiali contenuti nei numerose vasi excretori che si diramano nel parenchima del suo corpo." Finally, I quote from Mathias (1925, p. 20) as follows: "Le *T. tarda* Steenst. est la forme larvaire immobile d'un Holostomide, mais ce n'est pourtant pas un kyste comme c'est le cas général chez les Trématodes. En effet, un kyste véritable est caractérisé par une membrane d'enveloppe continue, hyaline, d'épaisseur variable, qui isole complètement la cercaire enkystée du milieu ambiant."

That this investiture of the mature *T. cornuta* is really a cyst was recognized by Henle (1835, p. 597), Diesing (1858, p. 366), De Filippi (1859, p. 215), Von Linstow (1894, p. 332) and Szidat (1924, p. 310). Faust (1917 a, p. 111), concerning *T. flabelliformis*, states that some of the parasites were encysted. My sections show clearly that the parasite is surrounded by a definite extra-cuticular membrane. A similar cyst has been described for *Adolescaria perla* Sinitsîn (a metacercaria parasitic in *Mytilus edulis* and *Venus sp.* in the Black Sea) by Sinitsîn (1911, p. 39) in the following words: "The cyst consists of a gelatinous, or even cartilaginous, membrane which lies close to the body of the *Adolescaria* with which it forms, technically speaking, a unit."[1]

Apertures in the cyst of *T. flabelliformis* adjacent to the excretory pore and the external openings of the oral and lateral suckers and the cavity of the hold-fast organ were not mentioned by Faust; such apertures, however, are clearly visible in the cotype specimen. An aperture adjacent to the excretory pore in the cyst of *T. cornuta* has been observed by many authors; Szidat (1924, p. 310, Fig. 8) also showed the presence of a canal in the wall of the cyst adjacent to the oral sucker.

[1] From a translation of the original Russian prepared under the direction of Professor Henry B. Ward by Mr. Alexis M. Bagusin, a Russian student of zoölogy at the University of Illinois.

The cyst is hyaline, non-cellular, and apparently of parasite origin; even when ruptured it adheres to the cuticle of the parasite and cannot be removed without injury to the latter. The wall of the cyst shrinks materially in the process of fixation, becoming a thin membrane (Fig. 1 *c*) closely applied to the surface of the parasite. This membrane does not interfere with staining *in toto*, differing in this respect, from the usual behavior of cysts of parasite origin. Whether the ability of stains to reach the parasite within the cyst depends entirely upon the presence of natural apertures in it, or, in part, upon its peculiar permeability, was not determined. The fact that the cyst wall shrinks in the process of fixation would seem to indicate that it is more or less permeable. In two living specimens, not under cover-glass pressure, the cysts were 0.018 mm. and 0.036 mm. thick.

THE PARASITE

Inasmuch as the present species closely resembles *Tetracotyle cornuta*, with which it may be identical and which has often been carefully described (see synopsis), it seems unnecessary to give a detailed account of its structure. Certain misinterpretations made by Faust, however, deserve consideration.

The eversion of the lateral suckers and the hold-fast organ in the form of lappets in the larva is doubtless an abnormal condition brought about by excessive imbibition of water, and not a property of maturity, as suggested by Faust (1917 b, p. 63, Pl. III, Fig. 40). In living specimens freshly removed from the host the walls of these cavities are never everted. In dead and water-soaked specimens, however, they are generally protruded.

The reproductive system is represented by a single median poorly differentiated mass of cells (Fig. 1 *rf*) located in the anterior part of the hind-body. The reproductive system, according to Faust (1917 b, p. 45), comprises ovary, oviduct, uterus, genital pouch, vitelline glands and testes fairly well developed. In reality most of the structures thus described are lobes of the irregularly shaped hold-fast organ (Fig. 1 *hf*) and the ramifications of the cavity (*chf*) among them. The acetabulum was considered to be a primitive genital pore (Faust, 1917 b, Pl. III, Fig. 38). Similar

misinterpretations made by Faust (1917 b, p. 64) concerning the reproductive system of *Tetracotyle pipientis* were corrected by Hughes (1928, p. 46).

The reserve bladder is similar to that described by Hughes (1927, p. 257) for a tetracotyle found in the pericardial cavity of *Stizostedion canadense griseum* De Kay[2] as "an irregular continuous coarse-meshed network of spaces, not in the form of definite vessels" filled with (p. 256) "a thin hyaline fluid, containing calcareous granules of various sizes, which move freely in *any direction* in response to pressure or movements of the body." The reserve bladder, therefore, does not comprise a regular system of definite vessels, as suggested by Faust (1922, p. 80), based on his description (Faust, 1917 b, p. 37, Pl. III, Fig. 39). Similar errors made by Faust (1917 b, p. 64) concerning the excretory system of *Tetracotyle pipientis* were corrected by Hughes (1928, p. 48). In addition to the granules freely moving in the spaces of the reserve bladder, others are found confined to more or less isolated pockets adjacent to the body-walls. These granules, however, less regular in shape and size than in *T. pipientis*, show a tendency to be arranged in groups of some 4 or 5 in a row, the ensemble of these groups constituting a very irregular subcuticular net-work. Oil droplets such as were found in *T. pipientis* (Hughes, 1928, p. 49) do not occur. A few flame cells and scattered tubules of the primary excretory apparatus were observed, but not in sufficient number and detail to give a definite idea of their arrangement.

Five living specimens from *Lymnaea stagnalis perampla*, freshly removed from the host and not under cover-glass pressure, varied in length from 0.298 mm. to 0.407 mm. (average 0.366 mm.) and in width from 0.255 mm. to 0.291 mm. (average 0.262 mm.). Faust (1917 a, p. 111) found that "mature" specimens were somewhat larger than these measurements indicate. He regarded as mature, however, specimens in which the lateral suckers were everted and which were therefore in an abnormally distended condition. The following measurements were determined from a few (1 to 3) living specimens under cover-glass pressure: oral

[2] A description of this tetracotyle is being presented in another paper of the present series.

sucker, from 0.054 mm. to 0.060 mm. in diameter; acetabulum, from 0.048 mm. to 0.060 mm. in length and from 0.057 mm. to 0.060 mm. in width; mouth of the cavity of the hold-fast organ (Fig. 1 *ohf*), from 0.015 mm. to 0.063 mm. (average 0.041 mm.) in width; external opening of lateral sucker (*ols*), 0.030 mm. in length. The principal measurements of ten preserved specimens mounted *in toto* are shown in the accompanying table.

TABLE I

PRINCIPAL MEASUREMENTS IN MILLIMETERS OF TEN *TETRACOTYLE FLABELLIFORMIS*, PRESERVED AND MOUNTED *IN TOTO*

	Minimum	Maximum	Average
Body, length	0.218	0.282	0.253
" width	0.162	0.212	0.191
Hind-body, length	0.070	0.095	0.081
" width	0.115	0.151	0.133
Oral sucker, diameter	0.042	0.053	0.047
Acetabulum, diameter	0.035	0.049	0.043
Hold-fast organ, diameter	0.077	0.098	0.086

DISCUSSION

The worms upon which this study has been made are regarded as being identical with *Tetracotyle flabelliformis* Faust, since in general form and size they conform to Faust's descriptions and in those details wherein they differ from his descriptions they agree with the cotype specimen. Furthermore, ample reasons have been given for considering certain statements in Faust's descriptions to be in error; these points have already been discussed.

A number of names for tetracotyles found in invertebrate hosts (snails and leeches) occur in the literature, but in general not enough is known of these forms to distinguish them from *Tetracotyle cornuta* (Rudolphi), the larva of *Strigea cornuta* (Rudolphi) according to Szidat (1927, p. 400). *Tetracotyle orientalis* Faust, demonstrated by Faust (1922, pp. 81, 82) to be the larva of *Cyathocotyle orientalis* Faust, constitutes an exception to this state-

ment. A tentative list of all known and supposed species of tetracotyles parasitic in invertebrate hosts is given in the following synopsis. Synopses of tetracotyles parasitic in fishes and other vertebrates are being presented in other papers of the present series.

SYNOPSIS OF TETRACOTYLES PARASITIC IN INVERTEBRATES

1. *Tetracotyle hirudinum* (Diesing) Mataré

SYNONYMY:

Cercarienformen, Distoma, cercarienartige — aus der Matrix des Blutegels	Henle (1835, pp. 587, 598, 608, respectively)
"Distom" (from) *Hirudo vulgaris L.*	Creplin (1841, p. 79)
Heptostomum hirudinum	Schomburgk (1844, p. 166)
Heptastomum Hirudinum Schomburgk	Diesing (1850, p. 418) *ex parte*
Heptastomum Hirudinum Schomburgk	Diesing (1858, p. 370)
Tetracotyle hirudinum	Mataré (1910, p. 496)
Tetracotyle (Heptastomum) hirudinum (Schomburgk)	Faust (1918 b, p. 75)

This form, first found and briefly described by Henle (1835, p. 587, Taf. XIV, Fig. 7a), was again briefly described by Diesing (1850, p. 418, and 1858, p. 370, Taf. II). Although Diesing's figures are poor and have the anterior and posterior ends of the worms confused, they show typical tetracotyliform structures. Monticelli (1888, p. 92), Brandes (1890, p. 578) and Braun (1892, p. 795) recognized *Heptastomum* to be a larval holostome; Von Linstow (1894, p. 330) considered it identical with *Tetracotyle cornuta*. It has been reported from *Nephelis vulgaris* and *Clepsine complanata*.

2. *Tetracotyle cornuta* (Rudolphi) mihi

SYNONYMY:

Distoma (from) *Planorbis corneus*	Henle (1835, p. 597)
Schmarotzer im Sporenschlauche der Cercaria armata und echinata	Von Siebold in Burdach (1837, p. 195), according to Diesing (1858, p. 366)
Distoma (of) *Cercaria armata Siebold*	Steenstrup (1842, p. 46)
Distoma tarda	Steenstrup (1842, p. 75), *ex parte*
Heptastomum Hirudinum Schomburgk	Diesing (1850, p. 418), *ex parte*

Tétracotyle	De Filippi (1855, p. 351)
Tetracotyle Limnaei	Pagenstecher (1857, p. 32)
Distoma tardum	Claparède (1858, p. 103)
Tetracotyle typica Diesing	Diesing (1858, p. 366), *ex parte*
Tetracotyle typica Dies. (*e. p.*)	Von Linstow (1877, p. 193)
Del Tetracotile De Filippi	Ercolani (1881, p. 284)
Tetracotyle tipica, Diesing	Leidy (1891, p. 416) ?
Tetracotyle tarda Steenst.	Mathias (1925, p. 16)
Tetracotyle tarda (Steenstrup) Mathias	Hughes (1927, p. 255)
Tetracotyle typica de Filippi	Szidat (1927, p. 401)

First found and briefly described by Henle (1835, p. 597), this tetracotyle has since been described in more or less detail by Steenstrup (1842, pp. 46, 76; Pl. III, Figs. 5–6), Von Siebold (1843 a, p. lviii, and 1843 b, p. 325), De Filippi (1855, p. 351; Pl. II, Fig. 25, and 1859, pp. 213–219; Pl. III, Figs. 24–31), Moulinié (1856; Pl. VII, Fig. 19), Pagenstecher (1857, p. 32; Taf. III, Figs. 15–18), Diesing (1858, p. 366), Ercolani (1881, p. 284, Tav. II, Figs. 16–18), Von Linstow (1894, p. 330, Taf. XXII, Figs. 1–8), Lühe (1909, p. 169), Faust (1918 b, p. 76), Szidat (1924, p. 306; Figs. 4–9) and Mathias (1925, p. 17; text Figs. 2, 3; Pl. I, Figs. 2–4; Pl. II, Figs. 1, 2, 4, 5, 6, 10). It has been reported from various species of *Lymnaea*, *Planorbis* and *Paludina* in Europe. In North America Leidy (1891, p. 416) and in South America Lutz (1921) have reported the occurrence of tetracotyles which they identified with the present species. In this synopsis these reports are discussed in connection with *Tetracotyle flabelliformis* and *T. molluscipeta*, respectively.

T. cornuta often occurs in, and associated with, the parthenitae of other trematodes, to which it was considered to be genetically related by Steenstrup (1845, p. 79) and De Filippi (1855, p. 352; 1857, p. 427; and 1859, p. 217). This idea, already opposed by Henle (1835), Von Siebold (1843 a, 1843 b), Moulinié (1856, p. 150) and Pagenstecher (1857), was shown to be entirely untenable by Ercolani (1881, p. 286). Ercolani (p. 288) further succeeded in demonstrating by feeding experiments that in ducks the tetracotyles develop into adult holostomes which he identified, in error, as *Holostomum erraticum* Dujardin. Von Linstow (1877, p. 195) studied the development of two-eyed miracidia from the eggs of *Holostomum cornucopia* Molin. A superficial resemblance between

these larvae and tetracotyles led him to suppose that tetracotyles develop directly from eggs. Supported by this assumption of Von Linstow and the work of Ercolani, Leuckart (1889, p. 163) advanced the theory that holostomes are "metastatic" trematodes, having an alteration of hosts but not of generations. This theory, accepted by Brandes (1890, p. 570) and Braun (1892, p. 796), held sway until Lutz (1921) discovered that tetracotyles develop from furcocercous cercariae produced in thread-like sporocysts parasitic in snails. The entire life-cycle of the tetracotyle was worked out by Mathias (1922 and 1925). He classified the adult as *Strigea tarda* Steenstrup. Szidat (1924) observed the penetration of his *Cercaria A* into *Lymnaea palustris* and carefully studied its transformation into *Tetracotyle cornuta*. Szidat (1927, p. 400) demonstrated that the adult form should be identified with *Strigea cornuta* (Rudolphi), described as *Amphistoma cornutum* by Rudolphi (1809, p. 343). That the reserve bladder of *T. cornuta* is similar to that of *T. flabelliformis* is suggested by two figures of Von Linstow (1894, Taf. XXII, Figs. 6–7). The structures referred to as "Plasmastränge" are probably spaces of the reserve bladder. The structures interpreted by De Filippi (1855, Pl. II, Fig. 25) to be branches of the intestinal caeca are probably parenchymal strands interspersed in the reserve bladder.

3. *Tetracotyle flabelliformis* Faust

Synonymy:

? Tetracotyle tipica, Diesing	Leidy (1891, p. 570)
Cercaria flabelliformis	Faust (1917 a, p. 111)
Cercaria (Tetracotyle) flabelliformis Faust	Faust (1917 b, p. 37)
Tetracotyle flabelliformis	Faust (1922, p. 80)

Leidy (1891, p. 570) was the first to report the occurrence of a tetracotyle parasitic in snails in this country. He identified specimens from *Lymnaea catascopium* and *Physa heterostropha* as *Tetracotyle tipica*. Faust (1917 a, p. 111, and 1917 b, p. 63) considered that the tetracotyles were produced by the parthenitae of other trematodes, in which and associated with which they were often found.

4. *Tetracotyle iturbei* Faust

Synonymy:

Redia of Schistosomum mansoni	Iturbe and González (1917, p. 10)

This form, parasitic in *Planorbis guadalupensis* in Venezuela, was first found and figured by Iturbe and González (1917, Figs. 1–10) as rediae of *Schistosomum mansoni*. This material was redescribed as *Tetracotyle iturbei* by Faust (1918 a, p. 109, and 1918 b). Through the courtesy of Professor H. B. Ward of the University of Illinois to Professor La Rue, I have had opportunity to examine cotype material of this parasite and I find it to be very similar to, if not identical with, *Tetracotyle flabelliformis*. In regard to generative organs Faust made errors comparable to those in his descriptions of *T. flabelliformis*, which are discussed in the first part of this paper. The probability that certain interpretations were erroneous was suggested by Szidat (1924, p. 311) and Ashworth and Bannerman (1927, p. 162).

5. *Tetracotyle molluscipeta* (Lutz) mihi

Synonymy:

"*Tetracotyle . . . typica*"	Lutz (1921, p. 128)

Lutz (1921, p. 126) found a furcocercous cercaria, which he called *Dicranocercaria molluscipeta*, produced by parthenitae parasitic in snails. The cercaria was described in general terms which applied also to two other closely related species. He found, however, that *D. molluscipeta* differed from the other two species in certain structural details and in that it chose to penetrate snails (species of *Physa*, *Planorbis* and *Spirulina*) to further its development. In these snails the worms developed into encysted larvae which he identified as *Tetracotyle typica* and which, when fed to ducks, became adult holostomes, identified by him as *Strigea tarda* (Steenstrup). The fact that *Anas moschata* in Brazil is known to harbor two species of holostomes, *Strigea sphaerocephalum* (Westrumb) and *Strigea graciliformis* Szidat, neither indigenous to Europe, suggests a possibility that the *Strigea* obtained by Lutz, in the experiment mentioned above, may not be

identical with any European species. For this reason the specimens identified by Lutz as *Tetracotyle typica* are here listed, provisionally, as a separate species, under the name of the cercaria from which they were developed. Lutz's discussion of this species, however, can hardly be regarded as a description.

6. *Tetracotyle bdellocystis* (Lutz) mihi.

Another of the three species of furcocercous cercariae mentioned in the preceding paragraph (Lutz, 1921, p. 126), *Dicranocercaria bdellocystis*, was observed to penetrate into leeches (species of *Haementeria* and *Hemiclepis*) in which it became a tetracotyle. A description of the tetracotyle was not given.

7. *Tetracotyle orientalis* Faust

This species, parasitic in *Vivipara lapillorum* Heude, near Peking, China, was described by Faust (1922, p. 79, Pl. IX, Fig. 1, and Pl. X). In general form it resembles *T. flabelliformis*, from which it differs in having the following characters: (1) primordia of vitellaria, (2) intestinal caeca, short, (3) cyst wall not adherent to cuticle (since the parasite moves "freely within the cyst"). A careful study was made of the excretory system, particularly of the *primary excretory apparatus*. By a feeding experiment it was determined that the tetracotyle is the larva of *Cyathocotyle orientalis* Faust, parasitic in ducks (*Anas boschas*).

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University of Michigan

LITERATURE CITED

Ashworth, A. W., and Bannerman, J. C. W. 1927. On a Tetracotyle (*T. phoxini*) in the Brain of the Minnow. Trans. Roy. Soc. of Edinburgh, 55 : 159–172. 8 text figs., 1 pl.

Brandes, G. 1890. Die Familie der Holostomiden. Zool. Jahrb., Abt. f. Syst., Geogr. Biol., 5 : 549–604. 3 pls.

Braun, M. 1892. Vermes. Bronn's Kl. u. Ord. d. Thier-Reichs, 4 (Abt. 1A. Lief. 18–27) : 561–816. 23 text figs., 14 pls.

Claparède, E. 1858. Über die Kalkkörperchen der Trematoden und die Gattung Tetracotyle. Zeitschr. f. wissensch. Zool., 9 : 99–105. 1 pl.

Creplin, F. C. H. 1841. Enthelminthologie, Endozoologie, Entozoologie. Allg. Encycl. d. Wissensch. u. Künste (Ersch & Gruber), Sect. 1, 35 : 76–83. Leipzig.

Diesing, K. M. 1850. Systema Helminthum. Vol. 1, 679 pp. Vindobonae.

—— 1858. Revision der Myzhelminthen. Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Kl., 32 : 307–390. 2 pls.

Ercolani, G. 1881. Dell' adamento della specie all' ambiente. Nuove ricerche sulla storia genetica dei trematodi. Mem. Accad. d. Sc., d. Ist. Bologna, (4) 2 : 239–334. 3 pls.

Faust, E. C. 1917 a. Notes on the Cercariae of the Bitter Root Valley, Montana. Journ. Parasitol., 3 : 105–123. 1 pl.

—— 1917 b. Life History Studies on Montana Trematodes. Ill. Biol. Monogr., 4 (1) : 7–120. 9 pls.

—— 1918 a. Studies on Illinois Cercariae. Journ. Parasitol., 4 : 93–110. 2 pls.

—— 1918 b. The Anatomy of *Tetracotyle iturbei* Faust, with a Synopsis of Described Tetracotyliform Larvae. Journ. Parasitol., 5 : 69–79. 1 pl.

—— 1922. Phases in the Life History of a Holostome *Cyathocotyle orientalis* nov. spec. with Notes on the Excretory System of the Larva. Journ. Parasitol., 8 : 78–85. 2 pls.

Filippi, P. de. 1855. Mémoire pour servir à l'histoire génétique des trématodes. Mem. d. R. Accad. d. Sc. Torino, (2) 15 (Cl. d. Sc. Fis. e. Mat.) : 331–358. 2 pls.

—— 1857. Deuxième mémoire pour servir à l'histoire génétique des trématodes. Ibid., (2) 16 (Cl. d. Sc. Fis. e. Mat.) : 419–442. 2 pls.

—— 1859. Troisième mémoire pour servir à l'histoire génétique des trématodes. Ibid., (2) 18 (Cl. d. Sc. Fis. e. Mat.) : 201–232. 3 pls.

Henle, F. G. J. 1835. Über die Gattung Branchiobdella und über die Deutung der innern Geschlechtstheile bei den Anneliden und her-

maphroditischen Schnecken. Arch. f. Anat., Physiol. u. wissensch. Med. (1835) : 574–608. 1 pl.

Hughes, R. C. 1927. Studies on the Trematode Family Strigeidae (Holostomidae) No. VI. A New Metacercaria *Neascus ambloplitis*, sp. nov. Representing a New Larval Group. Trans. Amer. Microsc. Soc., 46 : 248–267. 2 pls.

—— 1928. Studies on the Trematode Family Strigeidae (Holostomidae) No. VII. *Tetracotyle pipientis* Faust. Ibid., 47 : 42–53. 1 fig.

Iturbe, J., and González, E. 1917. The Intermediate Host of *Schistosomum mansoni* in Venezuela. Special Publ., Nat. Acad. Med. Venezuela, 7 pp. 2 pls. Caracas.

Leidy, J. 1891. Notices of Entozoa. Proc. Acad. Nat. Sci. Phila., 410–418.

Leuckart, K. G. F. R. 1889. Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten. Ein Hand- und Lehrbuch für Naturforscher und Ärzte. 2 Aufl. Band I, Abt. II. 897 pp. Leipzig.

Linstow, O. F. B. von. 1877. Enthelminthologica. Arch. f. Naturg., 43 : 173–198. 3 pls.

—— 1894. Helminthologische Studien. Jen. Zeitschr. f. Naturw., 28 (n. F. 21) : 328–342. 2 pls.

Lühe, M. 1909. Parasitische Plattwürmer. I. Trematoden. Brauer's Süsswasserfauna Deutschlands. Heft 17, 217 pp. Jena.

Lutz, A. 1921. Zur Kenntnis des Entwicklungszyklus der Holostomiden. Vorläufige Mitteilung. Centralbl. f. Backt. u. Parasitenk. (1. Abt. Orig.), 86 : 124–129.

Mataré, F. 1910. Über eine neue Tetracotyle im Hirn von *Phoxinus laevis*. Zeitschr. f. wissensch. Zool., 94 : 488–540. 3 text figs., 1 pl.

Mathias, P. 1922. Cycle évolutif d'un trématode holostomide (*Strigea tarda* Steenst.). C. R. Acad. d. Sci., 175 : 599–602. 7 figs.

—— 1925. Recherches expérimental sur le cycle évolutif de quelques trématodes. Bull. Biol. Fr. Bel., 59 : 1–124. 13 text figs., 4 pls.

Monticelli, F. S. 1888. Saggio di una morfologia dei trematodi. Tesi per ottenere la privata docenza in zoologia nella R. Università di Napoli. 130 pp. Napoli.

Moulinié, J. J. 1856. De la reproduction chez les trématodes endo-parasites. Mém. de l'Inst. Nation. genevois, 3 : 7–278. 6 pls.

Pagenstecher, H. A. 1857. Trematodenlarven und Trematoden. Helminthologischer Beitrag. 56 pp., 6 pls. Heidelberg.

Rudolphi, C. A. 1809. Entozoorum sive Vermium Intestinalium Historia Naturalis. Vol. II, 458 pp., 1 pl. Amstelaedami.

Schomburgk, O. 1844. *Heptostomum hirudinum*. Notiz. a. d. Geb. d. Nat.-

u. Heilk., 30:136. Abstract of paper read before Gesellsch. Naturforsch. Freunde zu Berlin.

Siebold, C. T. E. von. 1843 a. Bericht über die Leistungen im Gebiete der Anatomie und Physiologie der wirbellosen Thiere in dem Jahre 1842. Arch. f. Anat. Physiol. u. wissensch. Med., (1843): i-lxxxvii.

—— 1843 b. Bericht über die Leistungen im Gebiete der Helminthologei für 1842. Arch. f. Naturg., 9 (2): 289–335.

Siebold, C. T. E. von, in Burdach, C. F. 1837. Die Physiologie als Erfahrungswissenschaft. 2 Aufl., Vol. II. Leipzig.

Sinitsîn, D. F. 1911. Parthenogenetic Generation of Trematodes and Its Progeny in the Molluscs of the Black Sea (Russian). Mém. Acad. Sci. St. Petérsbourg, (8) 30: 1–127. 17 text figs., 6 pls.

Steenstrup, J. J. S. 1842. Om Fortplantning og Udvikling gjennen vexlende Generationsraekker en saeregen Form for Opfostringen i de lavere Dyrklasser. 76 pp. 3 pls. Kjøbenhavn.

—— 1845. On the Alternation of Generations; or, The Propagation and Development of Animals through Alternate Generations: a Peculiar Form of Fostering the Young in the Lower Classes of Animals. Translated from the German version of C. H. Lorenzen, by George Busk. 132 pp. London.

Szidat, L. 1924. Beiträge zur Entwicklungsgeschichte der Holostomiden. I. Zool. Anz., 58: 299–314. 9 figs.

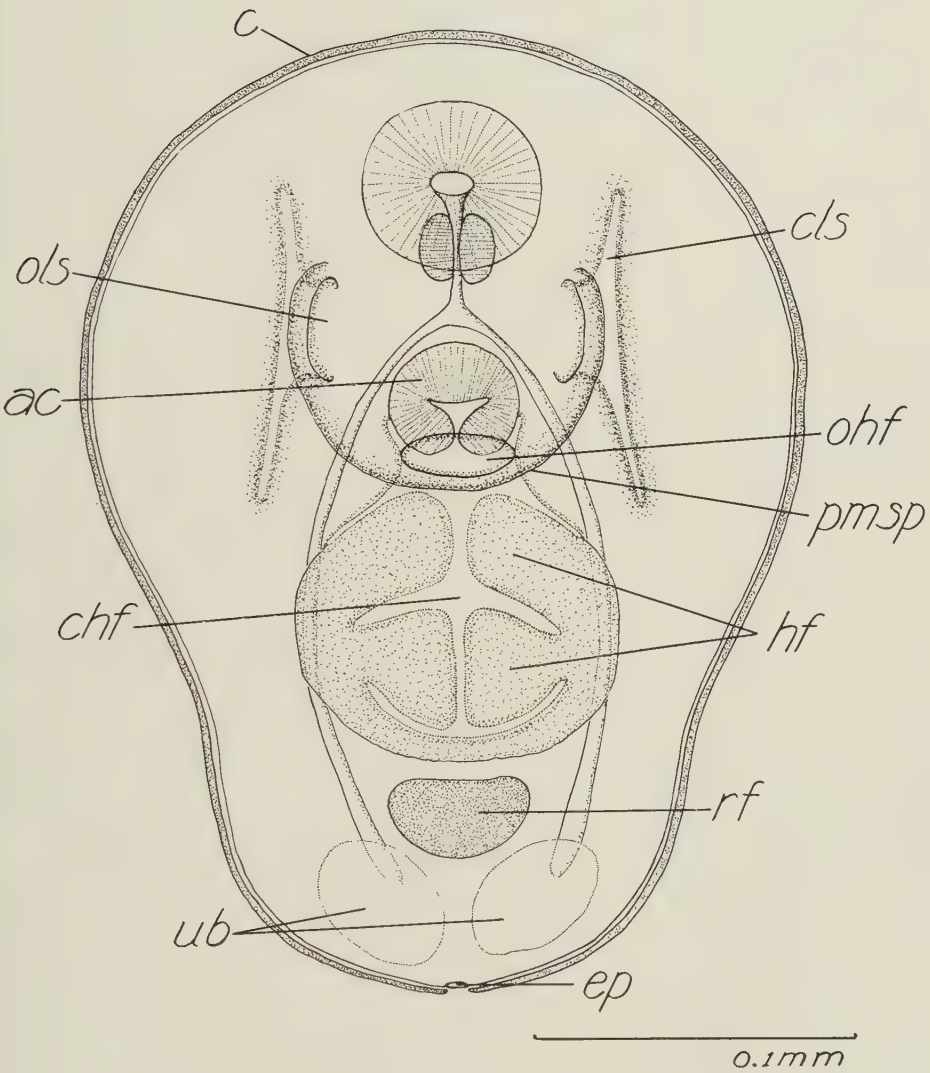
—— 1927. Die Parasiten des Hausgeflügels. Arch. f. Geflügelkunde, 1: 395–403. 4 figs.

EXPLANATION OF PLATE XXXIV

The following abbreviations are used: *ac*, acetabulum; *c*, cyst wall; *chf*, cavity of hold-fast organ; *cls*, cavity of lateral sucker; *ep*, excretory pore; *hf*, hold-fast organ; *ohf*, external meatus of hold-fast organ; *ols*, orifice of lateral sucker; *pmsp*, posterior margin of suctorial pocket; *rf*, fundament of reproductive organs; *ub*, urinary bladder.

Tetracotyle flabelliformis from *Lymnaea stagnalis appressa*, ventral view of a preserved specimen mounted *in toto*, drawn with the aid of a camera lucida. The digestive caeca, which are not visible in whole mounts, are added for the sake of clarity.

PLATE XXXIV



Tetracotyle flabelliformis Faust

STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. XVIII. *TETRACOTYLE SERPENTIS*, SP. NOV.

R. CHESTER HUGHES

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTOMIDAE) NO. XVIII *TETRACOTYLE SERPENTIS*, SP. NOV.*

R. Chester Hughes

University of Michigan, Ann Arbor, Michigan

An apparently new species of strigeid metacercaria, *Tetracotyle serpentis*, was found encysted in specimens of *Thamnophis sirtalis sirtalis* (Linné) taken in the vicinity of Douglas Lake, Cheboygan County, Michigan, during the summer of 1927.

The cysts of this parasite occur chiefly in the muscles of the host, a few in the adipose tissue about the mesenteries. Three of the 6 garter snakes examined were found to harbor 20, 19 and 3 tetracotyles respectively. Worms, apparently identical with the present species were also found in a water snake, *Natrix sipedon sipedon* (Linné). All of these snakes were heavily parasitized with two other species of strigeid larvae, namely, *Agamodistomum marcianae* (La Rue) and a diplostome, probably identical with *Cercaria vegrandis* La Rue.

In the study of the present species I have used the same methods and technique as that outlined in my paper (Hughes 1928: 43) on *Tetracotyle pipientis* Faust.

The elongate hyalin cyst, thin-walled and easily opened without injury to the parasite, is chiefly of parasite origin, the outer portion comprising a relatively thin layer of connective tissue. Twelve cysts, containing living parasites and free from cover-glass pressure, varied in length from 0.405 mm. to 0.525 mm. (average 0.473 mm.) and in width from 0.270 mm. to 0.375 mm. (average 0.294 mm.). The cavities of the same cysts varied in length from 0.375 mm. to 0.435 mm. (average 0.405 mm.).

The present species closely resembles *Tetracotyle pipientis*, with which it may be identical, and a detailed description is not necessary. *Tetracotyle serpentis* differs, however, from *T. pipientis* as described by Hughes (1928) as follows. It is somewhat smaller. The hind-body (fig. 1 *hb*) is larger and more elongate. The lateral suckers (*ls*) are less prominent and have their external openings located nearer to the oral sucker (*os*) and directed antero-ventral rather than postero-mesad. The acetabulum (*ac*) is relatively larger, broader than long in preserved specimens, and located farther from the anterior end of the body. The anterior lobes of the hold-

* Contribution from the Biological Station and the Zoological Laboratory, University of Michigan. This is the eighteenth of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

fast organ (*alhf*) are relatively larger. The pharynx is distinct from the oral sucker and clearly visible in specimens mounted *in toto*. The oesophagus, plainly visible in sections, bifurcates at a considerable distance anterior to the acetabulum. And lastly the spaces of the reserve bladder are not filled with large droplets of oil, although a few small droplets occur.

Seven living specimens of *T. serpentis*, free from the cyst and under cover-glass pressure, varied in length from 0.390 mm. to 0.540 mm. (average 0.462 mm.) and in width from 0.240 mm. to 0.375 mm. (average 0.296 mm.). In 5 living specimens under cover-glass pressure the oral sucker varied in diameter from 0.066 mm. to 0.105 mm. (average 0.073 mm.); and in 6 specimens the diameter of the acetabulum varied from 0.081 mm. to 0.111 mm. (average 0.094 mm.). The principal measurements of ten preserved specimens mounted *in toto* are given in the following table.

PRINCIPAL MEASUREMENTS IN MILLIMETERS OF TEN PRESERVED SPECIMENS OF *Tetracotyle serpentis* MOUNTED *in toto*.

	Minimum	Maximum	Average
Body, length	0.276	0.349	0.325
width	0.167	0.204	0.182
Oral sucker, diameter	0.042	0.053	0.047
Acetabulum, length	0.042	0.070	0.055
width	0.063	0.098	0.073
Hold-fast organ, length	0.057	0.133	0.092
width	0.070	0.095	0.080

The arrangement of the spaces of the reserve bladder and the position of the suctorial pocket are shown in cross-section in Fig. 2 (*srb*, *sp*). When the hold-fast organ is everted, as it frequently is, even in specimens freshly removed from the cyst, it extends forward in the suctorial pocket ventral to the acetabulum as two pairs of elongate lobes, the posterior lobes ventral to the anterior. In the fully extended condition the ends of these lobes project outside the suctorial pocket. The posterior lobes are somewhat the larger and in the process of eversion of the hold-fast organ they are the first to protrude from the suctorial pockets. In the everted hold-fast organ the tips of the posterior lobes are broad and blunt, whereas, the anterior lobes are tapering and pointed.

Synopses of the species of tetracotyliform larvae parasitic in invertebrates and in fishes are being presented in other papers of the present series. The species of tetracotyles which have been found in vertebrates other than fishes are as follows.

Synopsis of Other Tetracotyles Found in Vertebrates other than Fishes.

In Reptilia

1. *Tetracotyle strigis* (Schrank) mihi

Synonymy: *Tetracotyle Colubri* von Linstow (1877: 192)
Tetracotyle colubri Braun (1890: 572)

Brief descriptions of this form, based on the original description of von Linstow (1877: 192; Taf. XIII, fig. 22), have been published by Lühe (1909: 171; Fig. 123) and Faust (1918: 76). Two figures, but not a description, were given by Brandes (1890: Taf. XLI, figs. 12, 13). In these descriptions and a report by Sonsino (1893: 185) the worm has been listed from *Tropidonotus natrix* and *Pelias berus* in Europe. *Tetracotyle strigis* is about the same size as *T. serpentis*; otherwise the descriptions of *T. strigis* are too brief to enable one to make a careful comparison of the two species. The presence of a suctorial pocket in *T. strigis* is not indicated in any of the figures. The structure labelled "ovarium" in Brandes' Fig. 13 is doubtless the hold-fast gland.

Brandes (1890: 574) performed an experiment in which 6 *T. strigis* were fed to an owl, *Otus vulgaris*. The owl had been fed during a long period in captivity only on horse flesh and was presumed, therefore, to be free from strigeid parasites. About a month after the feeding of the tetracotyles the owl died and was found to contain one sexually mature holostome which Brandes described as *Diplostomum spathulaeforme*. According to Braun (1894a: 682, 1894b: 166 and 1894c: 755), however, feeding experiments which were more critically performed by the Ehrhardt brothers indicate that this larva becomes *Holostomum variabile* Nitzsch [*Strigea strigis* (Schrank)] in *Ulula aluco* and *Buteo vulgaris*. This parasite, according to Stiles and Hassal (1908: 269) was first described by Goeze (1782: 174; Pl. 14, figs. 4–6) and named *Festucaria strigis* by Schrank (1788: 16). The name of the tetracotyle should therefore be *Tetracotyle strigis* (Schrank).

2. *Tetracotyle tiliquae* Nicoll

This form, briefly described by Nicoll (1914: 349), occurs encysted beneath the abdominal peritoneum of the blue-tongued lizard, *Tiliqua scincoides*, in North Queensland, Australia. Through the courtesy of Dr. G. M. Heydon of Townsville to Professor La Rue, I have had an opportunity to examine three cotype specimens of *Tetracotyle tiliquae*. Unfortunately these specimens had been preserved in the cyst and it was not possible to make good preparations of them. Since Nicoll's description is very brief and no figure of the worm has been published, I am unable to determine whether this form may not be a diplostome rather than a tetracotyle (*sensu stricto*).

In Amphibia

3. *Tetracotyle crystallina* (Rudolphi) von Linstow

Synonymy: *Distoma crystallinum* Rudolphi (1819: 100) ex parte
Distomum crystallinum Rudolphi Diesing (1850: 352) ex parte
Distomum crystallinum Rudolphi Diesing (1858: 338)
Distomum crystallinum Hannover (1864: 3)
Tetracotyle crystallina Rud. von Linstow (1877: 194)
Tetracotyle crystallina (Rud) Braun (1892: 796)
Tetracotyle cristallina R. Sonsino (1893: 188)
Tetracotyle (*Distoma*) *crystallina* (Rud.) Faust (1918: 75)

This parasite was first described by Rudolphi (1819: 381). It has since been described by Dujardin (1845: 453), Diesing (1850: 352 and 1858: 338), Pagenstecher (1857: 39; Taf. IV, fig. 6), Lühe (1909: 170) and Faust (1918: 75). Bettendorf (1897: 311; Taf. 28, fig. 4) made a careful study of the muscle cells and fibers of this species. It was discussed in comparison with *T. pipientis* Faust by Hughes (1928: 51). It has been reported from *Rana temporaria, R. esculenta, Bufo viridis, B. igneus* and *Vipera berus*. Possibly the specimens found in *Vipera* are identical with *Tetracotyle strigis*.

4. *Tetracotyle pipientis* Faust

This form, from mesenteric cysts in *Rana pipiens* Schreber, was described by Faust (1917: 64; Pl. IV, figs. 47, 48, and 1918: 79) and redescribed by Hughes (1928).

5. *Tetracotyle gyrinipeta* (Lutz) mihi

Lutz (1921) found a furcocercous cercaria, which he named *Dicranocercaria gyrinipeta*, produced by thread-like sporocysts parasitic in snails. The cercaria was briefly described in general terms which applied also to two other closely related species. He found, however, that *D. gyrinipeta* differed from the other two species in minor morphological details and in that it chose to penetrate tree-frog larvae for further development. All stages in the transformation of the cercaria into a tetracotyle were observed but the mature metacercaria was not described. It is possible that Lutz used the name *Tetracotyle*, in a broad sense, to include all holostome larvae having lateral suckers, and that the species in question is a diplostome, rather than a tetracotyle (*sensu stricto*).

In Mammalia

6. *Tetracotyle soricis* von Linstow

Tetracotyle Soricis von Linstow (1877: 191)
Tetracotyle soricis v. Linst. Braun (1892: 797)

This form, found encysted in the connective tissues in *Sorex vulgaris* was briefly described by von Linstow (1877: 191). A description based on that of von Linstow was published by Faust (1918: 76). No figure of the species has been published.

7. *Tetracotyle foetorii* von Linstow

Tetracotyle Foetorii von Linstow (1876: 1)
Tetracotyle foetorii v. Linst. Braun (1892: 796)

This parasite, briefly described by von Linstow (1876: 1; Taf. 1, fig. 2) was found in *Foetorius putorius* encysted under the skin of the neck, between the neck muscles and on the oesophagus. According to the figure the intestinal caeca are short and the body is constricted anterior to the acetabulum. A description based on that of von Linstow was published by Faust (1918: 76).

In Aves

Species inquirendum

Tetracotyle sp. Lühe (1909: 171)

Tetracotyle from neck muscles of wild duck, Pagenstecher (1857: 246)
Tetracotyle typica Diesing, (1858: 367) ex parte

This parasite was found, but not described, by Pagenstecher (1857: 246). It may be identical with *Tetracotyle ardeae* Mataré (Mataré 1910: 496) which was described by Wedl (1857: 247; Taf. 1, fig. 8) as a "Geschlechtlich unenwickeltes Distoma" from species of Ardea, Gallinula and Podiceps and which according to the figure is probably not a tetracotyle at all.

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Literature Cited

Bettendorf, H. 1897.—Ueber Musculatur und Sinneszellen der Trematoden. Zool. Jahrb. (Abt. f. Anat.), 10: 307–538, 1 text fig., 5 pls.

Brandes, G. 1891.—Die Familie der Holostomiden. Zool. Jahrb., (Abt. f. Syst. Geog. u. Biol), 5: 549–604, 3 pls.

Braun, M. 1892.—Vermes. In Bronn's Kl. u. Ord. d. Thier-Reichs, Vol. IV. (Abt. I a Lief 18–27) pp. 561–816, 23 text figs., 14 pls.

1894a.—Helminthologische Notizen. IV. Zur Entwicklungsgeschichte der Holostomiden. Centralbl. f. Bakt. u. Parasitenk., 15: 680–682.

1894b-Zur Entwicklungsgeschichte der Holostomiden. Nach den Untersuchungen der Herren Alfred und Oscar Ehrhardt mitgetheilt. Zool. Anz., 17: 165–167.

1894c.—Zur Entwicklungsgeschichte der Holostomiden. Nach den Untersuchungen der Herren Alfred und Oscar Ehrhardt mitgetheilt. Zool. Centralbl., 1: 755.

Diesing, C. M. 1850.—Systema Helminthum. Vol. 1. 679 pp. Vendobonae.

1858.—Revision der Myzhelminthen. Abtheilung: Trematoden. Sitzungsb. d. k. Akad. d. Wissensch. Mat.—naturwissensch. Cl., 32: 307–390, 2 pls.

Dujardin, F. 1845.—Histoire naturelle des Helminthes ou vers intestinaux. 654 pp. Paris.

Faust, E. C. 1917.—Life history studies on Montana trematodes. Ill. Biol. Monogr., IV, 4: 1–120, 9 pls.

1918.—The anatomy of *Tetracotyle iturbei*, with a synopsis of described tetracotyliform larvae. Jour. Parasitol., 5: 69–79, 1 pl.

Hannover, A. 1864.—Iagttagelser over indkapslede indvoldsorme hos frøen. 15 pp. 2 pls. Kjøbenhavn.

Hughes, R. C. 1928.—Studies on the trematode family Strigeidae (Holostomidae) No. VII. *Tetracotyle pipientis*. Faust, Trans. Amer. Microsc. Soc., 47: 42–53, 1 fig.

Goeze, J. A. E. 1782.—Naturgeschichte der Eingeweidewürmer thierischer Körper. 471 pp. Blankenburg.

von Linstow, O. F. B. 1876.—Helminthologische Beobachtungen. Arch. f. Naturg., 42: 1–18, 2 pls.

1877.—Elminthologica. Idem., 43: 173–198, 3 pls.

Lühe, M. 1909.—Parasitische Plattwürmer. I. Trematoden. In Brauer's Süsswasserfauna Deutschlands. Vol. 17. 217 pp. Jena.

Lutz, A. 1921.—Zur Kenntnis des Entwicklungszyklus der Holostomiden. Centralbl. f. Bakt. u. Parasitenk. (1 Abt. Orig.) 86: 124–129.

Mataré, F. 1910.—Ueber eine neue Tetracotyle im Hirn von *Phoxinus laevis*. Zeitschr. f. wissensch. Zool., 94: 488–450, 1 pl.

Nicoll, W. 1914.—The trematode parasites of North Queensland. Parasitology, 6: 333–350. 2 pls.

Pagenstecher, H. A. 1857.— Ueber Erziehung des *Distoma echinatum* durch Fütterung. Arch. f. Naturg., 23: 244–251.

Rudolphi, C. A. 1819.—Entozoorum Synopsis. 811 pp., 3 pls. Berolini.

Schrank, F. v. P. 1788.—Verzeichniss der bisher hinlänglich bekannten Eingeweidewürmer nebst einer Abhandlung über ihre Anverwandtschaften. 116 pp. München.

Sonsino, P. 1893.—Trematodi di rettili e di anfibi della collezione del museo di Pisa. Atti Soc. tosc. di. sc. nat., Pisa. (Proc. verb.) 8: 183–190.

Stiles, C. W., and Hassal, A. 1908.—Index Catalogue of Medical and Veterinary Zoology. Trematoda and Trematode Diseases. U. S. Hyg. Lab. Bull., 37, 401 pp. Washington.

Wedl, K. 1857.—Anatomische Beobactungen über Trematoden. Sitzungsb. d. k. Akad. d. Wissensch. Math.—naturwissensch. Cl., 26: 241–278, 4 pls.

Abbreviations

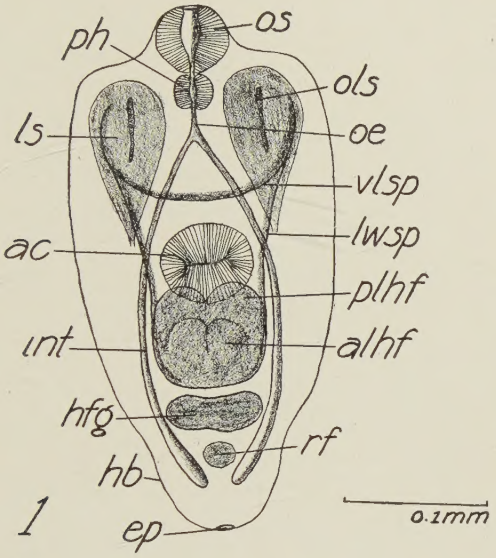
ac—acetabulum
alhf—anterior lobe of hold-fast organ
cmf—transverse muscle fibers
dvm—dorso-ventral muscle fiber
ep—excretory pore
hb—hind-body
hfg—hold-fast gland
int—intestinal caecum
lmf—longitudinal muscle fibers
ls—lateral sucker
lwsp—lateral wall of suctorial pocket
oe—oesophagus
ols—external opening of lateral sucker
os—oral sucker
ph—pharynx
plhf—posterior lobe of hold-fast organ
ps—parenchymal strand
rf—fundament of reproductive organs
sp—suctorial pocket
srb—spaces of reserve bladder
vlsp—antero-ventral lip of suctorial pocket

Plate III

Drawings made with the aid of a camera lucida.

Fig. 1. *Tetracotyle serpentis*, ventral view of a preserved specimen, mounted *in toto*. In this specimen the position of the oral sucker is somewhat unusual, the sucker being thrust forward and bent dorsally so that the mouth appears to be strictly terminal rather than ventro-terminal.

Fig. 2. *Tetracotyle serpentis*, cross-section through the anterior part of the acetabulum.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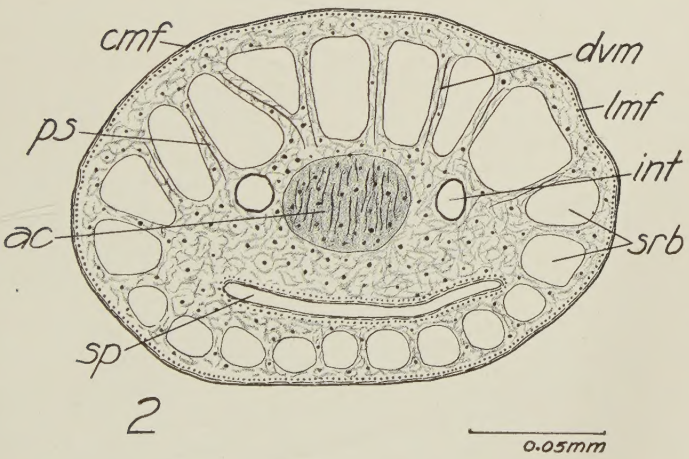
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PLATE III